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FINAL REPORT

ONR GRANT N00014-88-K-0387

July 1, 1988 - June 30, 1990

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EFFECTS OF FLUID ENVIRONMENT ON

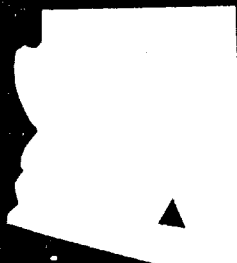
MICROBIAL UPTAKE KINETICS

Submitted to: Office of Naval Research

Submitted by: B. E. Logan, Assistant Professor
Department of Civil Engineering
and Engineering Mechanics

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90 10 01 114

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION Unclassified			1b. RESTRICTIVE MARKINGS		
2a. SECURITY CLASSIFICATION AUTHORITY			3. DISTRIBUTION/AVAILABILITY OF REPORT N/A		
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE					
4. PERFORMING ORGANIZATION REPORT NUMBER(S)			5. MONITORING ORGANIZATION REPORT NUMBER(S) N/A		
6a. NAME OF PERFORMING ORGANIZATION University of Arizona		6b. OFFICE SYMBOL (If applicable)		7a. NAME OF MONITORING ORGANIZATION N/A	
6c. ADDRESS (City, State and ZIP Code) Civil Engineering & Engineering Mechanics Building #72 Tucson, Arizona 85721				7b. ADDRESS (City, State and ZIP Code) N/A	
8a. NAME OF FUNDING/SPONSORING ORGANIZATION ONR		8b. OFFICE SYMBOL (If applicable)		9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER	
8c. ADDRESS (City, State and ZIP Code) Office of the Chief of Naval Research 800 N. Quincy Street Arlington, Virginia 22217-5000				10. SOURCE OF FUNDING NOS.	
11. TITLE (Include Security Classification) Effects of Fluid Environment on Microbial Uptake Kinetics				PROGRAM ELEMENT NO.	
				PROJECT NO.	
12. PERSONAL AUTHOR(S) Dr. Bruce E. Logan				TASK NO.	
13a. TYPE OF REPORT Final Scientific				13b. TIME COVERED FROM 7/01/88 TO 6/30/90	
14. DATE OF REPORT (Yr., Mo., Day) 90/9/26				15. PAGE COUNT	
16. SUPPLEMENTARY NOTATION					
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB. GR.			
19. ABSTRACT (Continue on reverse if necessary and identify by block number) <p>The purpose of this research project was to test our hypothesis that microbial uptake of large molecular weight compounds could be increased by fluid shear and advective flow past the microorganisms. This hypothesis was based on a biohydrodynamical uptake model derived from mass transfer and coagulation theories. In a series of experiments using both pure cultures of bacteria and natural assemblages of marine bacteria, we demonstrated that advective transport past bacteria increased uptake of low molecular weight compounds such as leucine. As predicted, fluid shear did not affect uptake of small molecular weight compounds with low diffusivities. However, fluid shear did increase uptake by suspended bacteria of large molecular weight compounds. Enhanced uptake was only observed when microbial transport systems were not saturated, since concentration gradients must be present at the cell surface in order for the fluid mechanical environment to affect uptake kinetics. These results imply that under nutrient limited conditions, attachment to detritus or other marine surfaces could increase bacterial uptake if there is sufficient advective flow past the cell. - R. J. -</p>					
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT UNCLASSIFIED/UNLIMITED <input checked="" type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS <input type="checkbox"/>			21. ABSTRACT SECURITY CLASSIFICATION N/A		
22a. NAME OF RESPONSIBLE INDIVIDUAL Randall S. Albarte			22b. TELEPHONE NUMBER (Include Area Code) (202) 696-4590		22c. OFFICE SYMBOL ONR/Code 1122B

FINAL TECHNICAL REPORT

ONR CONTRACT N00014-88-K-0387

"EFFECTS OF FLUID ENVIRONMENT ON MICROBIAL UPTAKE KINETICS"

Principal Investigator: B.E. Logan

INTRODUCTION

The purpose of this research project was to investigate the effect of small scale fluid mechanics on microbial uptake of low and high molecular weight dissolved organic compounds. Our hypothesis was that microbial uptake of large molecular weight compounds, under specific conditions, could be increased by fluid shear and advective flow past the microorganisms. This hypothesis was based on a biohydrodynamical uptake model derived from mass transfer and coagulation theories (Logan and Hunt 1987,1988). From equations used to describe mass transfer to microbes, and coagulation of microbes with substrate, it was predicted that substrate uptake could be increased by microorganisms in high shear environments versus microorganisms in nearly stagnant flow. However, the relative advantages of growth within high shear environments was calculated to increase with increasing molecular weights of organic matter. Thus, for low molecular weight compounds, increased uptake with fluid shear should be undetectable. The majority of uptake studies reported in the marine literature have focused on easily degraded compounds with small molecular weights, such as glucose and amino acids, despite research which indicates large molecular weight organics can account for a substantial fraction of dissolved organics. Therefore, researchers would not have observed an effect of fluid shear on uptake.

Experiments were proposed to study microbial uptake in two different fluid environments: suspended in fluid shear and fixed in a uniform flow field. The plan of study included: (a) defining pure culture microbial uptake as a function of substrate size and fluid environment using radiolabeled substrates to validate the biohydrodynamic

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SUMMARIZED RESEARCH RESULTS

INCREASED MASS TRANSFER TO MICROORGANISMS WITH FLUID MOTION

The effect of fluid flow and laminar shear on bacterial uptake was examined in the laboratory under a wider range of conditions than could be experienced by unattached and attached marine bacteria. During the first phase of research, we examined leucine uptake by pure cultures of suspended (unattached) bacteria (Z. ramigera). Since our mathematical analysis assumed bacterial transport systems were not saturated, we first examined the kinetics of leucine uptake by this bacterium. We measured leucine uptake at three different incubation times, at concentrations between $0.5 \mu\text{g l}^{-1}$ to 100 mg l^{-1} . Leucine uptake was linear with concentration over a range spanning 6 orders-of-magnitude. As incubation times increased, the rate of uptake decreased suggesting feedback from an intracellular leucine pool. For incubation times of 0.5, 1 and 7 minutes, the slopes ($\pm\text{SD}$) of uptake versus concentration (on log-log axes) were 7.0 ± 0.02 , 6.4 ± 0.02 , and $5.2 \pm 0.03 \times 10^{-3} \text{ min}^{-1}$, respectively. The r^2 values for all lines were greater than 0.98. These results indicate that saturation kinetics were not exhibited by Z. ramigera over a wide range of leucine concentrations.

Several flow experiments conducted with Z. ramigera held on $0.2 \mu\text{m}$ polycarbonate filters indicated leucine uptake by cells fixed in a flow field was 55-65% greater than leucine uptake by suspended cells. In these experiments, leucine uptake increased with fluid velocity at velocities less than $\sim 1 \text{ mm s}^{-1}$. Little or no increase in leucine uptake was observed at higher velocities between $1\text{-}2.5 \text{ mm s}^{-1}$. On occasion, some cells were completely inactivated at higher flow velocities and did not take up any leucine, probably due to cell disruption at vacuums of 600 mm Hg needed to obtain higher velocities. Fluid velocities below 1 mm s^{-1} were obtained at vacuums below 250 mm Hg, a range indicated by Fuhrman and Bell (1985) to prevent significant release of amino acids from marine microorganisms.

Fluid flow experiments using 0.45 cellulose acetate and 0.2 μm aluminum oxide filters to hold bacteria were characterized by large variations in bacterial uptake about mean values. Cellulose acetate filters retained large concentrations of [^3H]leucine label during flow experiments which could not be removed by filter rinsing. This substantially increased the amount of abiotic adsorption of leucine to filters. These increased background leucine concentrations produced large variations in uptake for a given fluid velocity. Based on average uptake, the effect of fluid motion on uptake was similar to that observed with the polycarbonate (surface) filters. A maximum average increase of 60% was consistent with previous results using the polycarbonate filters. However, the larger errors associated each measurement did not result in a significant correlation of fluid velocity with uptake.

The effect of fluid motion, predicted from a mass transfer analysis, was compared with experimental results. Mass transfer calculations were based on the assumptions that leucine concentrations at the cell surface were much less than the bulk concentration, that uptake was not zero-order (saturated), and that flow past cells was completely uniform. At a flow velocity of 1 mm s^{-1} , three different mass transfer correlations predicted increases 22, 65 and 110%, versus the observed increase of 55-65%. The correlation used by Munk and Riley (1952) consistently overestimated the effect of fluid motion. The mass transfer correlation developed by Logan and Alldredge (1989) gave the best-fit to the experimental results of this study.

The effect of fluid shear on leucine uptake by *Z. ramigera* was examined by suspending cells in a laminar shear field generated using a rotating cylinder device constructed for this research. Within experimental error, fluid shear did not increase leucine uptake by bacteria. Moreover, there was a consistent trend of decreasing uptake with shear that suggested that higher laminar shear rates impaired leucine uptake by suspended bacteria. This result confirmed the prediction of the mass transfer analysis indicates that fluid shear should not increase leucine uptake by more than 5% at shear rates below 50 s^{-1} .

Conclusions: From both experimental results and theoretical considerations, fluid shear is not expected to substantially increase microbial uptake by unattached bacteria. Uptake experiments with *Z. ramigera* showed that fluid shear, and therefore, fluid mixing intensity, did not increase leucine uptake by bacteria suspended in a laminar shear field. Fluid flow past bacteria, however, can substantially increase bacterial uptake. Leucine uptake by *Z. ramigera* increased by an average of 55-65% at flow rates up to 1 mm s⁻¹. In order for cells to be present in this type of flow field under natural conditions, they must either be attached to a surface or attached to other cells in permeable aggregates. We believe the effect of fluid motion on bacterial kinetics is attributable to compression of the concentration boundary layer surrounding surface transport proteins. The rate of leucine uptake suggests that binding proteins used to transport leucine into the cell can occupy approximately 1% of the cell surface area. A detailed description of this research is contained in Logan and Dettmer (1990).

INCREASED UPTAKE OF DISSOLVED ORGANICS BY MARINE BACTERIA AS A FUNCTION OF FLUID MOTION

The purpose of this investigation was to examine if fluid motion affected uptake by natural assemblages of marine bacteria in the same manner as a pure culture. Fluid motion should only increase uptake when uptake is not already saturated and at the maximum rate. We measured the uptake of two model compounds (leucine and glucose) by marine bacteria fixed in a uniform flow field and suspended in a laminar shear field.

All samples were collected from surface waters in the Roosevelt Inlet on the Delaware Bay (Lewes, DE) during August, 1989 through the assistance of David L. Kirchman (University of Delaware, Lewes). Surface samples were gravity-filtered through 0.8 μ m Nucleopore filters to remove bacterivores and autotrophs. Samples were either immediately used in experiments or kept overnight (aged) in the dark at room temperature. Leucine and glucose uptake by bacteria was determined using 4,5-[³H]leucine and [³H]glucose.

Fluid motion was predicted to increase microbial uptake only if transport were not saturated. During our initial experiments, leucine transport was probably saturated in surface waters of Roosevelt Inlet since the leucine concentration was 45 nM (August 7) and K_s for leucine uptake is probably <1 nM. To reduce leucine concentrations and obtain non-saturated uptake kinetics, we aged the bacterial size fraction ($<0.8 \mu\text{m}$ filtrate) for 24 hr in the dark; in the absence of leucine input from other microorganisms, bacterial uptake reduced leucine concentrations to 1.7 nM. Leucine concentrations in particle-free seawater were near the detection limit (<1 nM), indicating that the filtered water used for the flow experiments was not contaminated.

At low leucine concentrations (aged samples), we found that fluid flow past cells increased $[^3\text{H}]$ leucine uptake by a factor of 8 compared to uptake at low fluid velocities. $[^3\text{H}]$ Leucine uptake increased from $0.65 \text{ pg ml}^{-1} \text{ min}^{-1}$ at 3 m d^{-1} to a maximum of $5.4 \text{ pg ml}^{-1} \text{ min}^{-1}$ at $\sim 20 \text{ m d}^{-1}$. At higher fluid velocities, $[^3\text{H}]$ leucine uptake decreased to an average of $4.5 \text{ pg ml}^{-1} \text{ min}^{-1}$ for fluid velocities between $20\text{--}70 \text{ m d}^{-1}$. The maximum rate of leucine uptake in flow experiments was 63% less than the average uptake of suspended cells ($14.5 \pm 0.7 \text{ pg ml}^{-1} \text{ min}^{-1}$, $\pm\text{SD}$). Reduced uptake by cells on the filter was observed in other experiments.

To test our assumption that enhanced activity required unsaturated uptake kinetics, we repeated the above flow experiment, but added radiolabeled leucine at a concentration expected to saturate uptake kinetics. The concentration of leucine in aged samples for this experiment was 1.1 nM, and was below detection (<1 nM) in bacteria-free samples. When $10.8 \text{ nM } [^3\text{H}]$ leucine was added to the bacteria-free sample used in the flow experiments, no increase in uptake was observed with fluid motion. This confirmed our hypothesis that unsaturated kinetics was a necessary prerequisite for enhanced microbial uptake with fluid motion.

We also examined the effect of fluid motion on $[^3\text{H}]$ glucose uptake using aged samples from the Roosevelt Inlet. We were unable to detect any increase in fluid motion on uptake. $[^3\text{H}]$ glucose uptake by suspended averaged $3.9 \pm 0.3 \text{ pg ml}^{-1} \text{ min}^{-1}$, when

glucose was added at a concentration of 1 nM. At fluid velocities between 8-48 m d⁻¹, glucose uptake was an average of 3.1±0.4 pg ml⁻¹ min⁻¹, or about 23% lower than suspended samples.

At shear rates of 0-2.1 s⁻¹, fluid shear did not appreciably affect leucine uptake of either fresh or aged samples by more than ±10%. These results are consistent with previous pure culture experiments and mass transfer calculations for small molecular weight compounds with large diffusion coefficients. The lack of an effect of fluid shear arises from the inability of shear to significantly alter transport rates of chemicals to the cell surface compared to transport due to diffusion.

The area covered by outer membrane proteins involved in leucine transport (leucine porters) is important for examining the impact of flow on leucine utilization and for understanding how these bacteria survive in the low organic concentrations of seawater. We estimated that as much as 9% of the surface area of free-living marine bacteria was covered by leucine porters. This percent coverage is close to the surface area of porins, which are abundant nonspecific pores or channels found in Gram-negative bacteria (e.g. *E. coli*). Assuming that each *E. coli* cell contains approximately 10⁵ porins with a surface area of 0.9 μm² (Nikaido and Vaara 1987) and a total cell surface area of 6.7 μm², approximately 13% of the cell surface is occupied by porins. The surface area of specific leucine porters in *E. coli* is probably much less than for marine bacteria, although we are unaware of any published estimates.

Conclusions: Fluid motion can substantially increase uptake by microorganisms, but this effect is a function of several factors, including the substrate type and concentration. Marine bacteria held on filters in a flow stream of 10 m d⁻¹ assimilated leucine 8 times faster than bacteria under similar conditions in the absence of fluid motion. This enhancement was only observed when leucine uptake was not saturated. In order for fluid motion to increase uptake, concentration gradients must be present at the cell surface. At high leucine concentrations (~11 nM), fluid motion had no effect on leucine uptake probably because of the lack of a concentration boundary layer. These

results imply that under nutrient limited conditions, attachment to detritus could increase bacterial uptake if there is sufficient advective flow past the cell. A detailed description of this research is contained in Logan and Kirchman (1990).

THE POTENTIAL FOR INCREASED UPTAKE BY FLOCCULATING DIATOMS

Blooms of chain-forming diatoms commonly flocculate into centimeter-sized aggregates of living, vegetative cells following nutrient depletion in surface waters off southern California. We examined the hypothesis that diatom cells within aggregates experience increased nutrient uptake relative to unattached cells. We used data on aggregates collected from surface waters in the Santa Barbara Channel. Three species of diatoms, *Chaetoceros radicans*, *Ch. debilis*, and *Nitzschia sp.*, comprised $86 \pm 4\%$ of the cells examined. The remaining cells were classified as centrics, pennates, or dinoflagellates. Aggregates were very loosely held together by tangled spines.

Measured settling velocities of diatom flocs *in situ* ranged between 49 and 190 m d^{-1} , with an average of $110 \pm 40 \text{ m d}^{-1}$. Predicted settling velocities of the 12 diatom flocs ranged between 73 and 120 m d^{-1} and were not statistically different (t-test) from the observed settling velocities of these flocs, even though the calculated velocities did not appreciably increase with aggregate diameter. The average predicted settling velocity of $100 \pm 12 \text{ m d}^{-1}$ did not differ significantly from the *in situ* settling velocity of 110 m d^{-1} . Similar values for the observed and predicted settling velocities supported the calculation of an equivalent aggregate radius used in this analysis, and the application of the permeable aggregate model for diatom flocs.

Measured sinking velocities were used to calculate intra-aggregate flow velocities between 24 and 160 $\mu\text{m s}^{-1}$ ($2\text{--}14 \text{ m d}^{-1}$), with an average intra-aggregate velocity of 70 $\mu\text{m s}^{-1}$ (6 m d^{-1}), or about 5% of the observed aggregate settling velocity for 12 diatom flocs. A wide range of intra-aggregate velocities of $60\text{--}160 \mu\text{m s}^{-1}$ are predicted

for aggregates in a narrow size range of 17-20 mm in diameter. This reflects the variable porosity, and therefore, permeability, of diatom flocs.

For 12 diatom flocs, we calculated Relative Uptake Factors between 1.0-2.1 (average of 1.4), indicating that diatoms within the aggregate could potentially utilize dissolved nutrients up to 2.1 times faster than unattached diatoms experiencing fluid shear in the water column. This analysis accounts for nutrient depletion within the aggregate, and requires that both unattached diatoms and the diatom floc are exposed to low nutrient concentrations where a first order (unsaturated) uptake model accurately describes nutrient uptake kinetics. For these calculations, we assumed unattached diatoms existed in laminar fluid shear ($G=1 \text{ s}^{-1}$).

Relative Uptake Factors were also calculated assuming unattached cells sank in undisturbed fluid. Assuming a sinking velocity of 1 m d^{-1} for unattached cells, the Relative Uptake Factors of cells within aggregates ranged between 1.1 and 2.2. This suggests that consideration of unattached cells as either sinking or suspended within a laminar shear field is unimportant in obtaining Relative Uptake Factors greater than unity for diatom flocs.

Our data suggest that the flocculation of diatom blooms may be immediately adaptive because aggregation alters the fluid environment of a cell. A single unattached cell or chain is contained within a microscale eddy and must move with the bulk fluid; however, fluid flow around and through a rapidly sinking aggregate actually alters the fluid environment of associated cells, and increases nutrient uptake by attached cells compared to unattached cells. Flocculation may, therefore, be advantageous to nutrient-stressed diatoms by increasing the potential for uptake of scarce nutrients following an intense phytoplankton bloom.

Conclusions: Using permeability-porosity relationships, we calculated intra-aggregate flow velocities of $20\text{-}160 \mu\text{m s}^{-1}$ through diatom aggregates. Although subject to considerable uncertainty, a Relative Uptake Factor analysis, based on mass transfer equations, indicated that diatoms fixed within aggregates undergoing

gravitational settling could take up nutrients up to 2.1 ± 0.4 times faster than unattached diatoms experiencing laminar shear. Increased nutrient uptake by aggregated diatoms may be important in understanding the reasons for diatom floc formation. A detailed description of this research is contained in Logan and Alldredge (1989).

ENHANCED UTILIZATION OF MACROMOLECULES BY SUSPENDED BACTERIA IN SHEARED FLUIDS

The purpose of this final aspect of research was to investigate the effect of fluid shear on microbial uptake of high molecular weight dissolved organic compounds. We examined the effect of shear on uptake of two model macromolecules, an albumin (65,000 amu) as an example of a well characterized protein, and a dextran (70,000 amu) as an example of a complex carbohydrate.

Pure cultures of microorganisms were grown in suspended culture using a macromolecule as a carbon source in a defined mineral salts media. We grew Z. ramigera cultures only on a single protein (bovine serum albumin). Since this culture was unable to grow on dextran, we used E. coli in dextran degradation studies. In order to prepare cultures for uptake studies, we processed each culture through a series of 3 centrifugation/concentration steps (5000 g) rinsing the cells with mineral salts media. Prior to uptake experiments, all cell suspensions were filtered through a 5 μ m filter to remove flocs.

We performed two types of uptake studies. In the first set of experiments, we used radiolabeled albumin and dextran. The albumin was labeled with ^3H using procedures adapted from Jentoft and Dearborn (1979). ^3H -dextran was purchased from SIGMA. The uptake study was carried out in 60-ml BOD bottles, and uptake was measured as filterable radioactivity using 0.2- μ m polycarbonate filters. We monitored label incorporated in the cells in cultures stirred with magnetic stir bars versus undisturbed cultures. We found that uptake of the label was 2-4 times as large in stirred cultures as in undisturbed cultures. In protein degradation studies, we monitored

the accumulation of label in a <10,000 size fraction using 1.5 ml centricon centrifuge membrane filters (Millipore Co.). We found that label accumulated in the <10,000 amu fraction faster than in the undisturbed sample, indicating increased breakdown of the macromolecules with fluid shear.

In the second series of experiments, we added higher concentrations of proteins and dextrans (~10 mg/l) to suspended cultures, and monitored dissolved oxygen concentration in mixed and undisturbed cultures. We found that the oxygen utilization rate of mixed cultures was larger than undisturbed cultures, in agreement with radiolabel experiments.

An alternative explanation for the increased uptake of oxygen and macromolecules is that through fluid shear, we injured cells and increased cell respiration rates. However, when glucose was added to cultures, uptake of ^3H -glucose and oxygen utilization rates of mixed and undisturbed cells was not significantly different. In addition, the uptake of ^3H -leucine was not affected by mixing, in agreement with the biohydrodynamical model that shear does not significantly affect uptake of small molecules with high diffusion coefficients.

Conclusions: Fluid shear can significantly increase the rate of macromolecule degradation, as indicated through enhanced accumulation radiolabel in the cell, enhanced accumulation of macromolecule breakdown products (<10,000 amu), and increased oxygen utilization rates of mixed cultures versus undisturbed cultures. A detailed description of this research can be found elsewhere (Confer, 1990). A manuscript for publication in Applied and Environmental Microbiology is in progress.

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A P P E N D I C E S

Increased Mass Transfer to Microorganisms with Fluid Motion

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Accepted for publication October 31, 1989

The effect of fluid flow and laminar shear on bacterial uptake was examined under conditions representative of the fluid environment of unattached and attached cells in wastewater treatment bioreactors. Laminar shear rates below 50 s^{-1} did not increase leucine uptake by suspended cultures of *Zoogloea ramigera*. However, leucine uptake by cells fixed in a flow field of $\sim 1 \text{ mm s}^{-1}$ was 55–65% greater than uptake by suspended cells. Enhanced microbial uptake with advective motion is consistent with mass transfer rates calculated using Sherwood number correlations. Advective flow increases microbial uptake by increasing collisions between substrate molecules and cells through compression of the concentration boundary layer surrounding a cell. The rate of leucine uptake suggests that binding proteins used to transport leucine into the cell can occupy approximately 1% of the cell surface area.

INTRODUCTION

Microbial adhesion to surfaces, such as rocks or plastic media in trickling filters, and to suspended inorganic particles and other microorganisms in activated sludge reactors, is crucial for the performance of all biological wastewater treatment processes.^{1,2} Although the environmental factors that induce and control microbial adhesion are not well understood, it is evident that permanent bacterial attachment requires the production of extracellular polymers.^{3,4} In a competitive environment, carbon and energy requirements for polymer synthesis must be less than the benefits received from attachment. This suggests that attachment results in increased nutrient availability.

In most wastewater treatment bioreactors, nutrient concentrations are low, and microorganisms grow at rates substantially less than their maximum. At these concentrations, diffusion of nutrients to suspended cells may limit nutrient uptake. Microbial attachment alters the fluid environment of a microorganism; while unattached bacteria must move with the bulk fluid, attached bacteria can have advective flow past their surface. Therefore, attachment may increase nutrient uptake by reducing the limitation of transport set by diffusion.

Unattached bacteria in both engineered and natural environments are substantially smaller than the Kolmogorov

microscale of turbulence and exist within an environment characterized as laminar shear. In bioreactors, the mean shear rate is calculated from the power input by fluid mixing and aeration and is estimated⁵ to be in the range of $90\text{--}220 \text{ s}^{-1}$, although shear rates may be 70 times higher near impellers.⁶ Natural systems, such as lakes and oceans, are characterized by much lower shear rates in the range of $< 0.1\text{--}10 \text{ s}^{-1}$.⁷ Shear rates would have to approach 10^6 s^{-1} to reduce the microscale of turbulence to the size of a $1\text{-}\mu\text{m}$ cell.

Attached microorganisms exist in a variety of environments. Microbes can form aggregates as well as attach to suspended particles, rising bubbles, and fixed surfaces. Biological aggregates are highly porous, and theoretical calculations^{8–10} and experimental evidence^{11–13} support fluid motion through these aggregates at velocities approaching the free field velocity. Flocs from an activated sludge process $0.5\text{--}1.5 \text{ mm}$ in longest length sink at rates of $0.2\text{--}2 \text{ mm s}^{-1}$ (ref. 14) and bubbles formed during bioreactor aeration rise at velocities up to $200\text{--}300 \text{ mm s}^{-1}$ (ref. 15) and may transport both cells and flocs. Marine snow particles, large amorphous aggregates that form in marine systems comprised of phytoplankton, bacteria, and other suspended organic matter, sink at velocities up to 4.5 mm s^{-1} .¹⁶ Fluid motion has been shown to increase nutrient uptake of phytoplankton,^{17–19} and Logan and Hunt^{8,9} have calculated that substantial fluid flow through highly porous aggregates can result in an overall increase in uptake kinetics of attached bacteria.

The purpose of this investigation is to quantify the effect of fluid flow and fluid shear on bacterial kinetics since these two fluid environments represent the range of fluid motions experienced by attached and unattached bacteria. In this study, the effect of fluid environment on microbial growth was calculated by comparing leucine uptake by bacteria suspended in undisturbed fluid with uptake by bacteria fixed in a uniform flow field and suspended in a laminar shear field. Leucine uptake was monitored since the rate of leucine incorporation by bacteria has been determined to be an index of protein synthesis for laboratory and natural assemblages of bacteria.²⁰ The measured effect of fluid motion on bacterial kinetics was compared to a

model based on an analysis of mass transfer to spheres in advective flow and laminar shear fields.

EXPERIMENTAL METHODS

Culture Conditions

Pure cultures of *Zoogloea ramigera* (ATCC 1100), a flocculant gram negative rod that attaches through production of cellulose fibrils, were used in all experiments. *Z. ramigera* is thought to be important in the formation of rapidly settling flocs in activated sludge wastewater treatment reactors.²¹ Long-term cultures were maintained on nutrient broth agar (Difco) and transferred to sterile liquid media for each experiment. Liquid media consisted of 1 g L⁻¹ of glucose in a mineral salt buffer (MSB), containing, per liter of water: 0.57 g NH₄NO₃, 0.5 g KH₂PO₄, 1 g K₂HPO₄, 0.2 g MgSO₄, 0.04 mg FeCl₃, and 0.02 mg vitamin B-12. All cells used in these experiments were grown in a shaker table, harvested during exponential growth, and prefiltered (to remove flocs) through a 8- μ m cellulose acetate filter (Millipore Corp.) at 100 mm Hg immediately before use. All studies were performed within several hours using samples isolated from the same suspended culture.

Leucine uptake by cells of *Z. ramigera* was determined using radiolabeled leucine (4,5-[³H]leucine, 60 Ci mmol⁻¹, ICN Laboratories), supplemented with unlabeled leucine, at final concentrations of 10⁻³–10² mg L⁻¹. All samples were analyzed using a Beckman 5801 liquid scintillation counter with H-number correction for sample quenching. Microorganisms were enumerated using acridine orange-epifluorescence direct count methods.²² Cells were approximately 0.8 μ m wide by 2 μ m long, equivalent in volume to a spherical cell with 0.7 μ m radius.

Effect of Fluid Flow

The effect of fluid motion on bacterial uptake was measured by comparing leucine uptake of suspended cells to leucine uptake by cells fixed in a flow field. Cells were maintained in a flow field by vacuum filtration onto a 25-mm filter (various types), supported by a 5- μ m cellulose acetate backing filter. These filters were supported by stainless steel bases and funnels in a 10-place vacuum box fitted with Teflon valves (Hoefer Scientific). Three different types of filters were used to capture bacteria: 0.2 μ m polycarbonate filters (Poretics Corp.), 0.2 μ m aluminum oxide filters (Anotech Corp.), and 0.45 μ m cellulose acetate filters (Gelman Corp.). The 0.2- μ m filters are surface filters, and cells were completely retained on the surface of the filter. The 0.45- μ m filter is a depth filter, and some penetration of cells into the filter matrix may have occurred.

Cells were fixed in a flow field by vacuum filtration of a solution consisting of 200- μ L of prefiltered cells with an absorbance of 0.42 in 2 mL of MSB. The filter was immediately rinsed with 2 mL of sterile MSB until only a light film of MSB solution remained on the filter (to prevent cell desiccation). Cell loadings on the filter, by projected

surface area, were less than 1%. A solution of sterile MSB containing $\sim 0.5 \mu\text{g L}^{-1}$ [³H]leucine was pulled through the filter at different velocities by varying the vacuum. Incubation times between 1 and 2 min were achieved by varying flow volumes. After fluid had been pulled through the filter, bacteria were immediately inactivated using 2 mL of 2% formalin. Filters were rinsed with an additional 5 mL of sterile MSB, withdrawn from the vacuum box, and combined with 10 mL of ACS scintillation cocktail (Amersham Corp.) for counting. In separate experiments, repeated washing of cells with fresh media did not reduce radiolabel concentration, indicating formalin addition did not lyse cells. Further addition of radiolabeled leucine after formalin addition resulted in no leucine uptake, indicating a loss of cell activity.

Uptake by suspended cells was determined by incubation of 200 μ L of prefiltered cells with 2 mL of MSB containing [³H]leucine for same length of time as the flow experiment. After incubation, cells were inactivated using formalin, and the solution was filtered, rinsed, and analyzed as described above. Both the flow and suspended-counts were corrected by subtraction of abiotic blanks prepared under identical conditions.

Effect of Fluid Shear

A defined laminar shear environment was obtained using a rotating cylinder apparatus.²³ The device consisted of two vertically aligned concentric acrylic cylinders separated by a 9-mm gap. The fixed inner cylinder was 8 cm in diameter and 15 cm high. The outer cylinder rotated at a fixed speed producing a uniform shear rate between the cylinders. The shear rate G was calculated²³ using

$$G = \frac{2\pi s}{60} \frac{2R_1 R_2}{(R_2^2 - R_1^2)} \quad (1)$$

where s is the rotational speed (rpm) and R_1 and R_2 are the inner and outer radii, respectively.

Prefiltered cells (2 mL) were combined with 500 mL of MSB containing [³H]leucine, and 400 mL of this solution was poured into the rotating cylinder annulus. The cylinder was started, and time zero samples were obtained by withdrawing three 10-mL samples from the remaining undisturbed 100-mL suspended-cell solution. After 15 min, three 10-mL samples were withdrawn from the rotating cylinder solution at a depth of 5 cm and from the undisturbed suspended-cell solution. Samples were combined with formalin solution (2% final concentration) and filtered using 0.2- μ m polycarbonate filters. Uptake was calculated as the difference between initial and final radioactivity of the filters.

KINETIC MODEL

Assuming Monod kinetics, the change in nutrient mass concentration with time, dC/dt , for a microbial culture is

$$\frac{-1}{N} \frac{dC}{dt} = \frac{\mu C_s}{Y(K + C_s)} \quad (2)$$

where N is the cell number concentration, C and C_s are the bulk and cell surface nutrient concentrations, respectively, μ is the maximum growth constant, k is the half saturation constant, and Y is a yield coefficient. Since C_s is not easily determined, the kinetic constants are usually calculated assuming $C = C_s$.²⁴ At low nutrient concentrations, $C \ll K$, and Equation (2) can be simplified to a first-order kinetic expression,

$$\frac{-1}{N} \frac{dC}{dt} = \frac{\mu C}{YK} \quad (3)$$

As written, Equation (3) is independent of fluid motion. The effect of fluid motion on microbial kinetics can be determined by describing nutrient utilization using a mass transfer analysis.^{8,9} For a suspended culture containing only free-living cells, the rate of nutrient utilization shown in Equation (2) is equal to the rate of mass transfer to each cell, Q , or

$$\frac{-1}{N} \frac{dC}{dt} = Q = k4\pi a^2(C - C_s) \quad (4)$$

where k is a mass transfer coefficient and a is the cell radius. Fluid motion increases microbial uptake through compression of the concentration boundary layer surrounding the cell.

The mass transfer coefficient can be written in terms of the dimensionless Sherwood number, $Sh = ka/D$, where D is the nutrient diffusivity. For a spherical cell in stagnant fluid, the Sherwood number is unity. Incorporating the Sherwood number into Equation (3) and assuming C_s is very small,

$$Q = 4\pi aD \text{ Sh } C \quad (5)$$

Several Sherwood number correlations have been proposed to account for the effect of fluid environment on the rate of mass transfer to spheres. At Reynolds numbers much less than unity ($Re = ua/\nu \ll 1$), Brian and Hales²⁵ correlated the Sherwood number, Sh_u , for a sphere fixed in a flow stream of velocity u , with the Peclet number, $Pe_u = ua/D < 10^4$, as

$$Sh_u = (1 + 0.48 Pe_u^{2/3})^{1/2} \quad (6)$$

Munk and Riley²⁶ determined the effect of sinking velocity on mass transfer to phytoplankton using

$$Sh_u = 1 + 0.5 Pe + 0.6 Pe^2 \quad (7)$$

The effect of fluid velocity on phosphorous uptake by the diatom *Thalassiosira fluviatilis*, determined by Canelli and Fuhs,¹⁸ was used by Logan and Alldredge¹⁰ to calculate the Sherwood number in water as

$$Sh_u = 1.45 Re^{0.57} \quad (8)$$

A Sherwood number for a sphere in laminar shear flow, Sh_G , was derived by Frankel and Acrivos²⁷ for $Pe = a^2G/D \ll 1$ as

$$Sh_G = 1 + 0.26 Pe^{0.5} \quad (9)$$

Since the Sherwood number is unity for a cell in stagnant fluid, the ratio of uptake by a cell in a fluid field or in laminar shear, Q , to uptake by a cell in undisturbed or stagnant fluid, Q_s , calculated using Equation (5), is

$$\frac{Q}{Q_s} = Sh \quad (10)$$

Therefore, the ratio of mass transfer to cells in these differing fluid environments to cells in stagnant fluid conditions can be determined using one of the Sherwood number correlations in Equations (6–9).

RESULTS

Suspended Cultures

The kinetic models presented in Equations (3–6) require nutrient uptake by microorganisms to be a linear function of concentration. We examined leucine uptake by suspended cultures of *Z. ramigera* at three different incubation times, at concentrations between $0.5 \mu\text{g L}^{-1}$ and 100 mg L^{-1} . As shown in Figure 1, leucine uptake was linear with concentration over a range spanning six orders of magnitude. As incubation times increased, the rate of uptake decreased, suggesting feedback from an intracellular leucine pool. For incubation times of 0.5, 1, and 7 min, the slopes (\pm standard error) of the lines shown in Figure 1 were 7.0 ± 0.02 , 6.4 ± 0.02 , and $5.2 \pm 0.03 \times 10^{-3} \text{ min}^{-1}$, respectively. The r^2 values for all lines were greater than 0.98. These results indicate that saturation kinetics were not exhibited by *Z. ramigera* over a wide range of leucine concentrations. A similar lack of saturation kinetics over a wide range of substrate concentrations was also observed by Rubin and Alexander²⁸ for phenol mineralization by natural bacterial

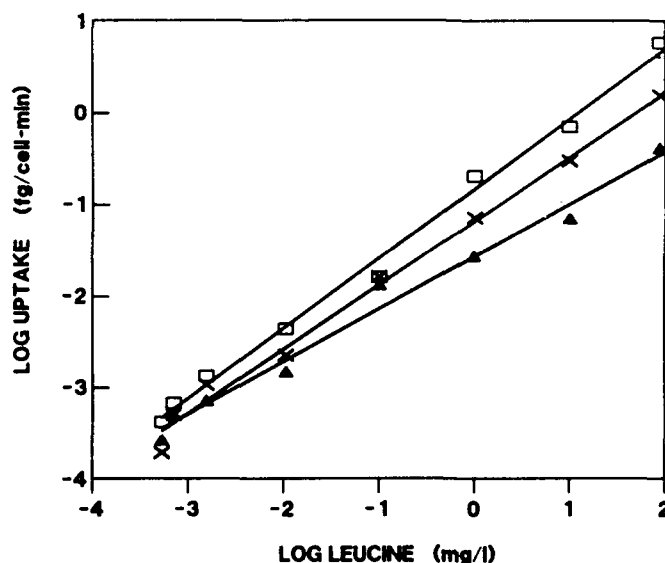


Figure 1. Leucine uptake as a function of leucine concentration, at different incubation times: (\square) 0.5 min, (\times) 1.0 min, and (\blacktriangle) 7 min. All lines have an r^2 value greater than 0.98.

assemblages at phenol concentrations between 0.002 and 200 $\mu\text{g L}^{-1}$.

Effect of Fluid Flow

Several flow experiments conducted with *Z. ramigera* held on 0.2- μm polycarbonate filters indicated leucine uptake by cells fixed in a flow field was 55–65% greater than leucine uptake by suspended cells. Shown in Figure 2 are two representative fluid flow experimental results. In the first experiment (Fig. 2a), only radiolabeled leucine was added to the culture. In the second experiment (Fig. 2b) cells were acclimated to 10 $\mu\text{g L}^{-1}$ of leucine for 30 min prior to addition of the radiolabel and the start of the experiment. In

these experiments, leucine uptake increased with fluid velocity at velocities less than $\sim 1 \text{ mm s}^{-1}$. Little or no increase in leucine uptake was observed at higher velocities between 1 and 2.5 mm s^{-1} . On occasion, some cells were completely inactivated at higher flow velocities and did not take up any leucine, probably due to cell disruption at vacuums of 600 mm Hg needed to obtain higher velocities. Fluid velocities below 1 mm s^{-1} were obtained at vacuums below 250 mm Hg, a range indicated by Fuhrman and Bell²⁹ to prevent significant release of amino acids from marine microorganisms.

Fluid flow experiments using 0.45 cellulose acetate and 0.2- μm aluminum oxide filters to hold bacteria were characterized by large variations in bacterial uptake about

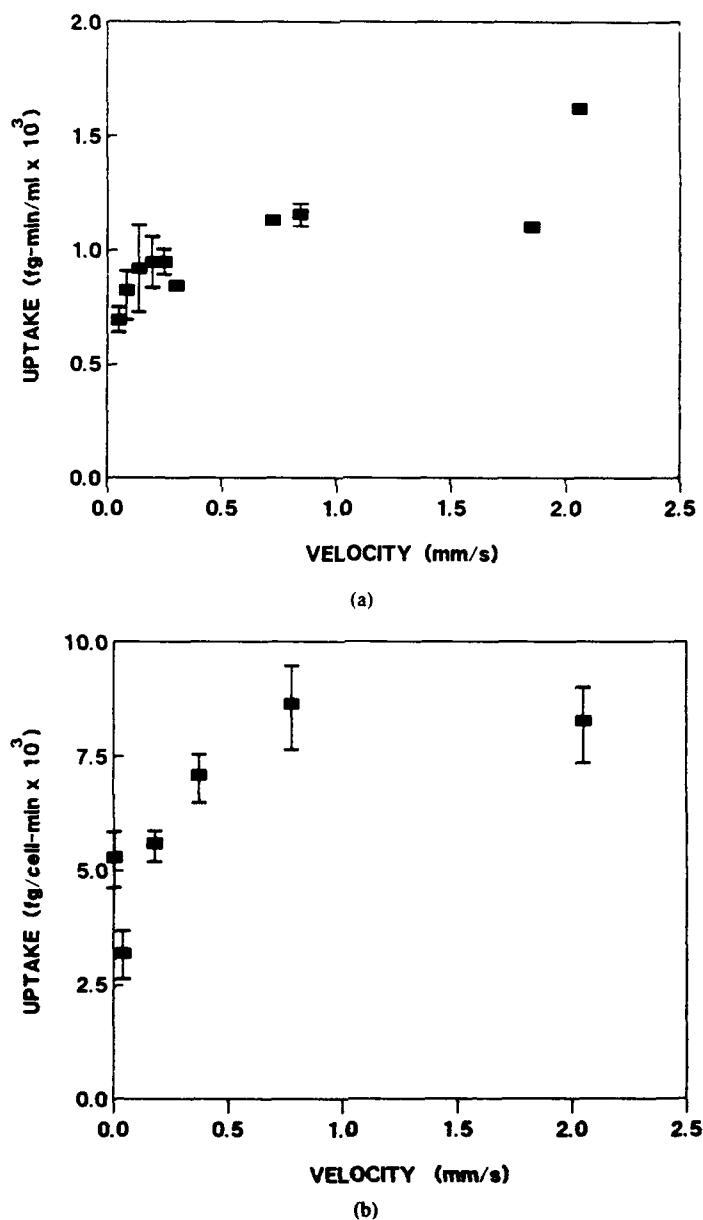


Figure 2. The effect of fluid velocity on leucine uptake for cells held on a 0.2- μm polycarbonate filter at different leucine concentrations: (a) 0.4 $\mu\text{g L}^{-1}$; (b) 10.8 $\mu\text{g L}^{-1}$. Lines are fitted by eye. Error bars correspond to ± 1 SD of 3–5 measurements.

mean values (Fig. 3). Cellulose acetate filters retained large concentrations of [^3H]leucine label during flow experiments which could not be removed by filter rinsing. This substantially increased the amount of abiotic adsorption of leucine to filters. These increased background leucine concentrations produced large variations in uptake for a given fluid velocity. Based on averages shown in Figure 3a, the effect of fluid motion on uptake was similar to that observed with the polycarbonate (surface) filters. A maximum average increase of 60% was consistent with previous results using the polycarbonate filters. However, the larger errors associated each measurement did not result in a significant correlation of fluid velocity with uptake.

The results shown in Figure 3b for the aluminum oxide filter suggest an increase in uptake with fluid flow, but the large variability of the data also do not present any significant pattern. Flow experiments with the aluminum oxide filters required much higher vacuums (>250 mm Hg) to achieve the same fluid velocities used in polycarbonate filter experiments. These high vacuums likely impaired net amino acid uptake.

The effect of fluid motion, predicted from a mass transfer analysis, is shown in Figure 4. Sherwood numbers were calculated using Equations (6–8), an average cell size of $0.7\text{ }\mu\text{m}$, and a molecular diffusivity for leucine of $0.7 \times 10^{-5}\text{ cm}^2\text{ s}^{-1}$ determined using the Wilke–Chang

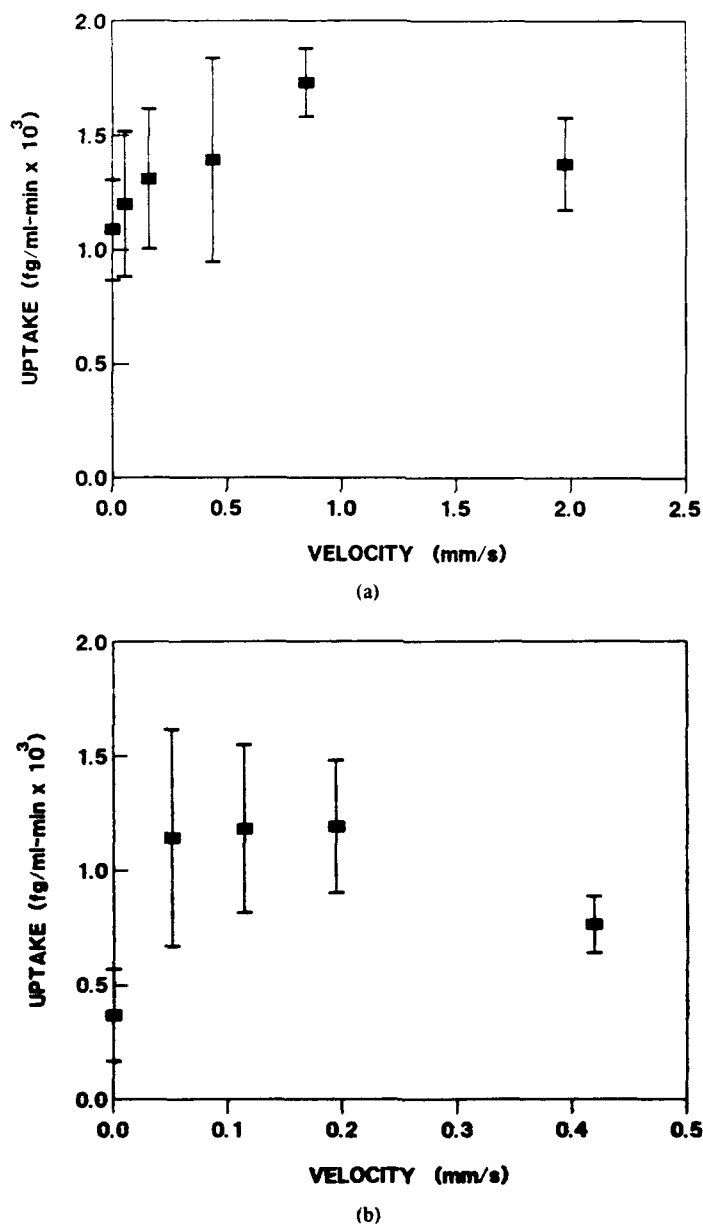


Figure 3. The effect of fluid velocity on leucine uptake for cells held on: (a) $0.45\text{ }\mu\text{m}$ cellulose acetate filter at $0.5\text{ }\mu\text{g L}^{-1}$ of leucine; (b) $0.2\text{ }\mu\text{m}$ aluminum oxide filter at $0.7\text{ }\mu\text{g L}^{-1}$ of leucine. Error bars correspond to ± 1 SD of 3–5 measurements.

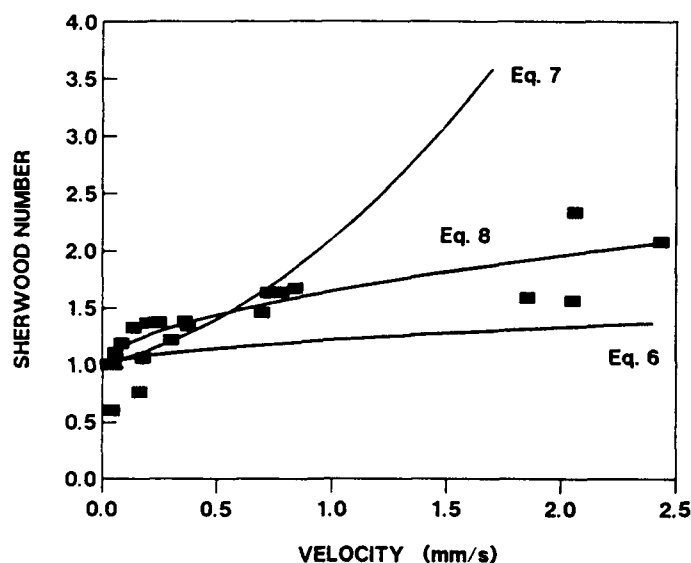


Figure 4. The effect of fluid velocity presented in terms of a Sherwood number. Data from Fig. 2 are plotted as a ratio of uptake by cells in a flow field to cells in stagnant fluid. Lines are plotted using Sherwood number correlations in Eqs. (6)–(8).

correlation.³⁰ These calculations were based on the assumption that leucine concentrations at the cell surface were much less than the bulk concentration, that uptake was not zero order, and that flow past cells was completely uniform. At a flow velocity of 1 mm s^{-1} , Equations (6), (7), and (8) predict an increase in mass transfer of 22, 110, and 65%, respectively. It can be seen in Figure 4 that the correlation used by Munk and Riley²⁶ consistently overestimated the effect of fluid motion. The mass transfer correlation developed by Logan and Alldredge¹⁰ [Equation (8)] gave the best fit to the experimental results of this study.

Effect of Fluid Shear

The effect of fluid shear on leucine uptake by *Z. ramigera* is shown in Figure 5 as a ratio of uptake by sheared cells to cells fixed in stagnant fluid. Although results at fluid shear rates above 50 s^{-1} are shown in Figure 5b, the fluid within the cylinder became unstable at these shear rates and could no longer be considered laminar. Values at zero shear were determined by not turning on the rotating cylinder after sample addition. This "zero shear" case was only within 20% of values obtained from analysis of suspended cultures. Thus, the experimental error of shear experiments is very large and at least 20%. Within this large experimental error, fluid shear does not increase leucine uptake by bacteria. Moreover, there is a consistent trend of decreasing uptake with shear that suggests that higher laminar shear rates impaired leucine uptake by suspended bacteria.

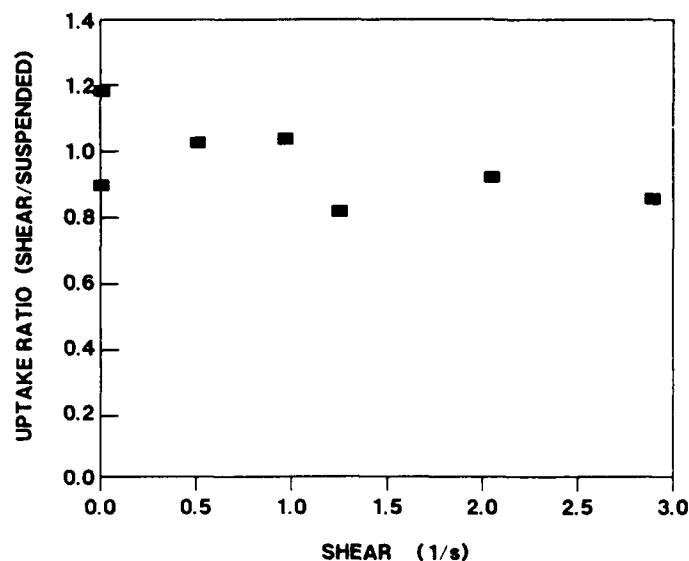
A mass transfer analysis indicates that fluid shear should not increase leucine uptake by more than 5% at shear rates below 50 s^{-1} . This is shown in Figure 6 using Equations (9) and (10) compared to data from Figure 5. The mathe-

matical analysis is limited to Peclet numbers much less than unity. For a cell radius of $0.7 \mu\text{m}$ and leucine diffusivity of $0.7 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, a Peclet number of 0.1 occurs at a shear rate of 140 s^{-1} and is larger than shear rates examined in this study.

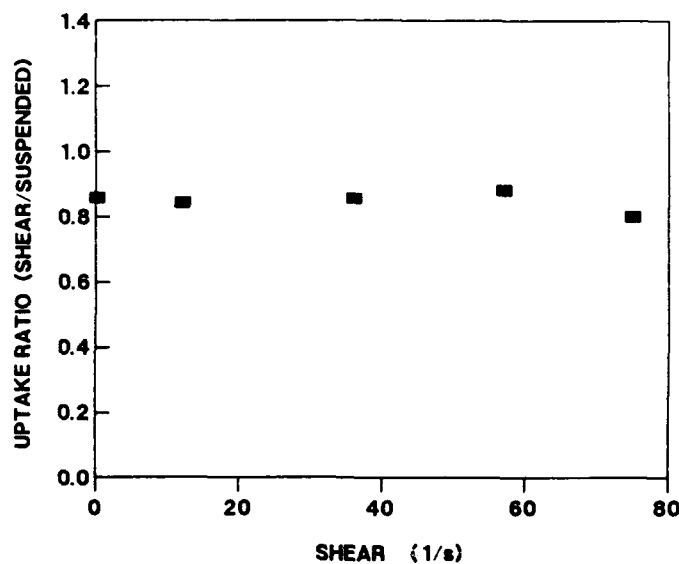
DISCUSSION

From both experimental results and theoretical considerations, fluid shear is not expected to substantially increase microbial uptake by unattached bacteria. Uptake experiments with *Z. ramigera* showed that fluid shear and, therefore, fluid mixing intensity, did not increase leucine uptake by bacteria suspended in a laminar shear field. Calculations based on a Sherwood number correlation [Equation (6)] predicted a maximum increase of only 5% at shear rates below 50 s^{-1} . This calculation is also in agreement with the prediction of Purcell³¹ that fluid shear at intensities typical of bioreactors could not substantially increase mass transfer of molecules to bacteria-sized particles.

Fluid flow past bacteria, however, can substantially increase bacterial uptake. Leucine uptake by *Z. ramigera* increased by an average of 55–65% at flow rates up to 1 mm s^{-1} . In order for cells to be present in this type of flow field under natural conditions, they must either be attached to a surface or attached to other cells in permeable aggregates. Enhanced chemical reaction rates with fluid motion have been demonstrated for forced air convection in a porous catalyst packed bed reactor^{32,33} and for hydrolysis of benzoyl L-arginine ethyl ester by CM-cellulose-ficin enzyme in a packed bed immobilized enzyme reactor.³⁴ Since we were able to show increased bacterial uptake with fluid motion, our experiments support predictions of enhanced uptake by cells within permeable aggre-



(a)



(b)

Figure 5. The ratio of leucine uptake by cells in a laminar shear field to uptake by cells in undisturbed fluid at (a) low and (b) high shear rates. Shear rates above 50 s^{-1} are no longer laminar.

gates for bacterial aggregates suspended in bioreactors and attached to bubbles.⁹

The proposed mass transfer model of bacterial uptake is analogous to a model proposed by Yao et al.³⁵ describing the filtration of molecules by particles (sand grains) in a water filter. The microorganism, like the inorganic filter particle, is a collector of material transported to the collector surface by Brownian diffusion. If the rate of molecular diffusion limits capture by the collector, then the rate of capture can be increased by advective fluid flow past the surface. This increase is attributed to a compression of the concentration boundary layer surrounding the collector which increases the mass flux to the collector surface.

The filtration model of Yao et al.³⁵ which uses a mass transfer analysis developed by Levich,³⁶ is based on evidence that molecules may collide many times with a surface before achieving a successful collision. The effect of unsuccessful collisions is incorporated in the filtration model using a parameter defined as a collector efficiency, composed of a probability of hydrodynamic encounter and a collision efficiency. An analogous argument is made here for the incorporation of a collector efficiency parameter into a microbial uptake/mass transfer model. According to the Michaelis-Menten description of enzyme kinetics, a molecule reversibly and repeatedly binds to an enzyme before successful product production. Very few enzymes

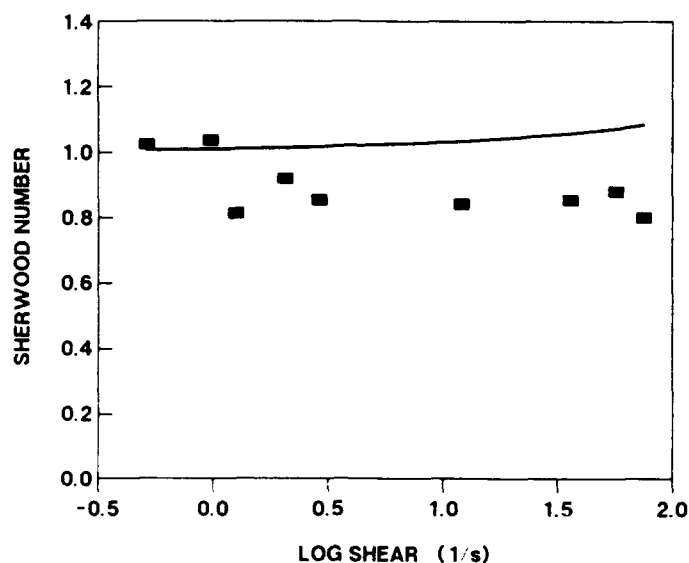


Figure 6. The effect of shear presented in terms of a Sherwood number. Data from Fig. 5, without zero values, are plotted as a function of the log shear rate. The line is plotted using the Sherwood number correlation in Eq. (9).

achieve "kinetic perfection,"³⁷ or operate at the limit of diffusion-controlled uptake rates. Therefore, the success of repeated molecule-enzyme binding, or collision efficiency, must be less than unity for microbial systems.

A collector efficiency can be calculated for leucine uptake using the experimental results of this study for suspended cultures of *Z. ramigera*. During leucine uptake, the bulk and surface concentrations of nutrient are related by

$$C_s = (1 - E_B)C \quad (11)$$

where E_B , the collector efficiency of a cell, is assumed to be a function of the extent of molecule-enzyme affinity as well as the shape of the cell. Substituting Equation (11) into Equation (4) and using data in Figure 2a, the resulting collector efficiency is approximately equal to 0.01. By this calculation, only once during every 100 collisions of a molecule with the cell is leucine incorporated into the cell.

The low value of the collector efficiency ($E_B \ll 1$) implies the concentration gradient surrounding a cell is negligible and that leucine uptake is kinetically controlled. If this were true, compression of the concentration boundary layer under these conditions would not increase bacterial uptake of leucine by more than 1%. However, this assumes complete coverage of the bacterial surface by binding proteins used for leucine transport across the bacterial membrane. Complete enzyme coverage on the cell surface for leucine transport is unlikely. The effect of incomplete enzyme coverage on mass transfer can be calculated using the analysis of Dudukovic and Mills.³⁸ For their analysis, the size of an enzyme must be known and is estimated here as 0.003 μm from size-molecular weight values given in Perry and Chilton³⁰ and assuming an enzyme molecular weight of 100,000. If the concentration boundary layer spans 0.01 μm , which would occur for a 1% change in

concentration across a 1- μm cell, then Dudukovic and Mill's analysis indicates the mass transfer coefficient must be reduced by the fraction of area available for leucine uptake. For example, if enzyme coverage is only 1% of the surface area and fluid motion reduces the concentration boundary layer to within 10% of the thickness under stagnant flow conditions, uptake by a cell fixed in a flow field will be 3.3 times as large as leucine uptake under stagnant flow conditions. Incomplete enzyme coverage of the cell can therefore explain the observed effect of fluid motion on bacterial uptake of leucine.

Other factors may have contributed to the observed effect of fluid motion on microbial uptake, and three additional explanations were examined. First, incomplete label dissolution during suspended-cell experiments could reduce leucine uptake. When Rhodamine dye was substituted for the added radiolabel, dissolution of the dye was nearly instantaneous, indicating sufficient mixing. Second, the vacuum pressures might have indirectly increased leucine uptake. However, very high vacuums (~ 600 mm Hg) frequently inhibited leucine uptake by cells and indicated a negative response of cells to vacuum. Other studies have shown high shear will inhibit the uptake of another amino acid by attached bacteria. Eighmy and Bishop³⁹ found that binding proteins used for aspartate uptake by mixed cultures biofilms were inhibited at shear rates of 3.1 N m^{-2} . Third, there may have been a slime capsule around the cell that reduced leucine transport to the cell surface. However, no noticeable capsule was observed on cells prefiltered through the 8- μm filter and used in these experiments.

Additional studies reported in the literature support the effect of enhanced uptake by attached bacteria and cells fixed in a flow field. Kirchman and Mitchell⁴⁰ and Simon⁴¹

have shown that bacteria attached to particles in natural waters such as lakes are larger and have higher uptake rates of amino acids than suspended bacteria. These studies were not well controlled with respect to fluid hydrodynamics and alternative explanations, such as colonization of particles by different species than those remaining unattached, and increased concentrations of amino acids adsorbed to particle surfaces, etc., could also explain their observations. However, a study by Canelli and Fuhs¹⁸ conclusively demonstrated the effect of fluid motion on phosphate uptake by pure cultures of the diatom *Thalassiosira fluviatilis* held on a filter surface. They observed a 130% increase in phosphorous uptake with fluid velocities up to 0.4 mm s⁻¹ and no further increase in uptake above this velocity. Based on their data, a collector efficiency of $E_B = 0.025$ was calculated for phosphate uptake by this diatom.⁹ Thus, an increase in uptake kinetics, similar to that observed in this study for uptake of an amino acid by bacteria, was observed by Canelli and Fuhs for a different cell and substrate than were examined in this study.

The results of the present study suggest that the effect of fluid motion may be adequately described by a dimensionless Sherwood number correlation. The collision of a molecule moving by Brownian motion with a collector, defined in the filtration model of Yao et al.³⁵ by the product of two terms, the probability of collision and the collision or sticking efficiency, are combined in this discussion in the constant E_B , the collector efficiency. For microorganisms, incomplete coverage of the cell surface by enzymes reduces the area available for mass transfer to the cell. Therefore, the difference in values of $E_B = 0.01$ calculated for leucine uptake by *Z. ramigera* and $E_B = 0.025$ determined for phosphate uptake by *T. fluviatilis* likely occurs due to the different affinities of the nutrient for the respective enzyme as well as incomplete surface coverage of enzyme. Advective flow past the cell either modifies the sticking efficiency of the molecule or, more likely, enhances the probability of collisions of nutrient with the cell surface.

Based on the results of this study, a model describing the rate of uptake by a cell as a function of fluid environment can be constructed by combining Equations (4) and (9) to obtain

$$Q = 4\pi aDE_B C_s Sh \quad (12)$$

In this model, the collision efficiency of the nutrient with the cell surface is limited to $0 \leq E_B \leq 1$. The Sherwood number has no physical limits, but high fluid velocities past the cell will ultimately damage the cell and decrease nutrient uptake. In our experiments, we typically observed a maximum increase of 55–65%, although further increases were limited by cell disruption at higher vacuums needed to provide higher fluid velocities. Microorganisms residing on the surface of pipes may reside in fluid with much higher velocities than examined here.⁴²

An increase in microbial uptake with fluid motion can be an important competitive advantage for attached cells in

biological wastewater treatment reactors. In activated sludge reactors, long detention times of cells imply low growth rates but do not require low uptake rates of easily degraded soluble organics. Under optimal conditions, attached microorganisms could utilize easily degraded organics up to 65% faster than unattached cells, assuming all cells had the same enzyme to assimilate the substrate. As a result, attached cells would outcompete unattached cells for growth substrates. The effect of fluid flow on microbial kinetics, therefore, may serve as a basis for understanding the advantages of microbial attachment and may help explain why most microorganisms are inherently capable of attachment.

Funding was provided by ONR Grant N00014-88-K-0387, and the donors of The Petroleum Research Fund, administered by the ACS. The authors appreciate comments by J. R. Hunt and D. L. Kirchman on the original manuscript and are grateful to P. Dahlen and D. B. Wilkinson for their assistance with laboratory experiments.

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Submitted to: Marine Biology
June 22, 1990

INCREASED UPTAKE OF DISSOLVED ORGANICS
BY MARINE BACTERIA AS A FUNCTION OF FLUID MOTION

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ABSTRACT

Fluid motion can increase the assimilation of dissolved organics by attached compared to free-living microorganisms. This increase results from compression of the concentration boundary layer surrounding the cell, and only occurs when uptake systems are not saturated. We examined the effect of advective flow and fluid shear on the uptake of model compounds (leucine and glucose) by natural assemblages of heterotrophic bacteria. We found that [^3H]leucine uptake by cells held in fluid moving at $20\text{--}70\text{ m d}^{-1}$ was 8 times larger than uptake by cells at a velocity of 3 m d^{-1} . This effect was only observed at low leucine concentrations ($\sim 1\text{ nM}$), when uptake was likely not saturated. When we added leucine at concentrations expected to saturate leucine uptake ($\sim 11\text{ nM}$), fluid motion past cells did not affect uptake. Fluid flow past bacteria did not increase [^3H]glucose uptake, and laminar shear rates of $0.5\text{--}2\text{ s}^{-1}$ did not increase either glucose or leucine uptake by suspended bacteria. These results indicate that fluid motion increases bacterial uptake of certain low molecular weight dissolved organics only when the microorganism exists in an advective flow field. As predicted from a mass transfer model, fluid shear rates in natural systems are too low to affect bacterial uptake of such compounds.

INTRODUCTION

The metabolic activity of heterotrophic bacteria attached to particles is higher than that of unattached bacteria in pelagic ecosystems. On a per-cell basis, uptake by attached bacteria is larger than uptake by free-living bacteria for glucose (Ibierri et al. 1982), glucose and glutamate (Kirchman and Mitchell 1982), dissolved ATP (Hodson et al. 1981), phosphate (Paerl and Merkel 1982), thymidine (Jeffery and Paul 1986), protein hydrosylate (Simon 1985), and amino acids (Bright and Fletcher 1983, Palumbo et al. 1984). Part of this difference can be attributed to the larger cell size of attached bacteria compared with free-living bacteria (Kirchman 1983, Alldredge et al. 1986; Simon 1987) or to utilization of the particle itself. However, these explanations cannot account for the observed increased activities of bacteria attached to inert surfaces compared with similarly-sized free-living cells (Bright and Fletcher 1983, Fletcher 1986).

The enhanced metabolic activity of attached bacteria may also be related to differences in fluid environments experienced by attached versus free-living bacteria. The microenvironment of free-living heterotrophic bacteria can be characterized as laminar shear. Unattached bacteria must move with the bulk fluid, and their cell size is substantially smaller than the Kolmogorov microscale of turbulence. Shear rates would have to approach 10^6 s^{-1} to reduce the microscale of turbulence to the size of a $1 \text{ }\mu\text{m}$ bacterium (Purcell 1978), which is unlikely in lakes and oceans where the highest shear rates in the upper few meters of waters may only reach $2\text{-}7 \text{ s}^{-1}$ (Soloviev et al. 1988). However, bacteria attached to detritus, such as marine snow, can have advective flow past their surface. Marine snow can be highly porous and can sink at velocities approaching 200 m d^{-1} (Alldredge and Gotschalk 1988). Theoretical calculations (Logan and Hunt 1987, 1988, Logan and Alldredge 1989) and experimental evidence (Logan 1987, Li and Ganczarczyk 1987; Wittler 1986) indicate that velocities of fluid motion through high-porosity aggregates approach the free-field velocity. Therefore, bacteria within these aggregates can reside in an advective flow field (Logan and Hunt 1987).

Fluid motion should affect bacterial uptake of dissolved organics analogous to how fluid motion affects phytoplankton. Nutrient uptake by phytoplankton increases with either fluid flow past the cell or with fluid shear (Canelli and Fuhs 1976, Pasciak and Gavis 1975, Mierle 1985). In laboratory experiments with pure cultures of bacteria, Logan and Dettmer (1990) showed that leucine uptake by Zoogloea ramigera fixed in a flow field of 1 mm s^{-1} was 55-65% greater than uptake by suspended cells. However, shear rates as large as 50 s^{-1} did not affect leucine uptake (Logan and Dettmer 1990). The effect of fluid flow on natural marine bacteria, which differ from those cultivated in the laboratory, has not been examined.

The purpose of this investigation was to examine if fluid motion affected uptake by natural assemblages of marine bacteria in the same manner as a pure culture and to examine the assumptions of a theoretical model developed to predict the effect of fluid motion on uptake. The model is based on the assumption that fluid motion enhances uptake by compressing the concentration boundary layer surrounding the cell (Logan and Hunt 1987). Therefore, fluid motion should only increase uptake when uptake is not already saturated and at the maximum rate. We measured the uptake of two model compounds (leucine and glucose) by bacteria fixed in a uniform flow field and suspended in a laminar shear field. We found that bacterial uptake of leucine, but not glucose, increased with the rate of flow and that the effect of fluid motion could be removed at elevated leucine concentrations. These results imply that under nutrient limited conditions, attachment to detritus could increase bacterial uptake if there is sufficient advective flow past the cell.

METHODS

Sampling sites and procedures: All samples were collected from surface waters in the Roosevelt Inlet on the Delaware Bay (Lewes, DE) during August, 1989. Surface samples were gravity-filtered through $0.8 \text{ }\mu\text{m}$ Nucleopore filters to remove bacterivores and

autotrophs. Samples were either immediately used in experiments or kept overnight (aged) in the dark at room temperature.

Uptake rates and concentrations: Dissolved free amino acids (DFAA) were measured by high performance liquid chromatography (HPLC, Rainin) using an OPA-reverse phase method (Lindroth and Mopper 1979) with a C18-reverse phase column. The analytical procedures were essentially those of Fuhrman and Bell (1985) with α -aminobutyric acid as an internal standard. Samples for HPLC analysis were filtered with Gelman Acrodiscs (25 mm with pore size of 0.45 μ m).

Leucine and glucose uptake by bacteria was determined using 4,5- 3 H]leucine (53 Ci mmol $^{-1}$, ICN Laboratories) and 3 H]glucose (18 Ci mmol $^{-1}$, ICN Laboratories). All samples were analyzed using a Beckman 3801 Liquid Scintillation Counter, with H-number correction for sample quenching. Microorganisms were enumerated using the acridine orange-epifluorescence direct count method (Hobbie et al. 1977).

The uptake rate of radiolabeled glucose and leucine by suspended bacteria in undisturbed fluid was determined prior to conducting experiments on the effects of fluid motion on uptake. Samples were spiked with radiolabeled leucine or glucose at the same concentrations used in flow experiments (\sim 1 or 11 nM). Samples (10 ml, in triplicate) were withdrawn at 0, 2 and 10 min, combined with formalin (2% final concentration) and vacuum filtered at 250 mm Hg through 0.2 μ m nylon filters (Poretics Corp.). Filters were rinsed twice with 5 ml of filtered seawater and radioassayed.

Effect of Fluid Flow: The effect of fluid motion on bacterial uptake was examined by measuring uptake of leucine or glucose by cells that were held on the surface of a filter. Filter-bound bacteria were prepared by filtration of the <0.8 - μ m size fraction (10 ml) through a 0.2 μ m nylon filter (Poretics Corp.) supported by a 5 μ m cellulose acetate backing filter (Millipore Corp.). These filters were held by stainless steel bases and funnels (25 mm diameter) in a 10-place vacuum box fitted with teflon valves (Hoefer Scientific). The nylon filter is a depth filter, and some penetration of

cells into the filter matrix may have occurred. Based on cell size and filter area, we calculate that cells covered less than 1% of the filter surface.

Flow past bacteria was obtained by pulling particle-free seawater past the filter-bound bacteria. Particle-free media was prepared by addition of radiolabeled leucine or glucose to either fresh or aged seawater that had been previously filtered through a 0.2 μm polycarbonate filter (Poretics Corp.). This solution was applied to the filter-bound bacteria with a syringe with a 0.2 μm in-line filter (Gelman Corp.). Different fluid velocities were obtained by varying the vacuum; incubation times were kept between 1-2 minutes by varying flow volumes. After all the fluid had been pulled through the filter, bacteria were immediately killed using 2 ml of 2% formalin, and rinsed with an additional 10 ml of 0.2- μm filtered seawater. Filters were added to 7 ml of scintillation cocktail (Aquasol II) and radioassayed.

Effect of Fluid Shear: A defined laminar shear environment was obtained using a rotating cylinder apparatus (van Duuren 1968). Our device consisted of two vertically aligned concentric acrylic cylinders separated by a 9 mm gap. The fixed inner cylinder was 8 cm in diameter and 15 cm high. The outer cylinder rotated at a fixed speed producing a uniform shear rate between the cylinders.

Radiolabeled substrates were added to 500 ml of the aged bacterial size fraction ($<0.8 \mu\text{m}$), and 400 ml of this solution was poured into the rotating cylinder annulus. The cylinder was started, and time zero samples were obtained by withdrawing three 10-ml samples from the remaining undisturbed 100 ml. After 10 min the rotating cylinder was stopped and the inner cylinder withdrawn. Three 10-ml samples were withdrawn from the rotating cylinder and from the undisturbed bacterial size fraction. Formalin (2% final concentration) was added and the sample was filtered through 0.2 μm nylon filters. Uptake was calculated as the difference between initial and final radioactivity of the filters.

Arsenate inhibition of leucine and glucose uptake: To begin to determine whether or not leucine and glucose are transported by similar mechanisms, the effect of

arsenate on uptake was examined. The monopotassium salt of arsenic acid (Sigma) was added directly to an aged <0.8 μm size fraction to avoid leucine or glucose contamination. After addition, the seawater was mixed and incubated for 5 min. [^3H]leucine or [^3H]glucose was then added and uptake followed (in duplicate) over time. Filters were rinsed once with 4 ml of filtered seawater and radioassayed. Controls did not receive any arsenate.

Kinetic model: The effect of fluid motion on microbial kinetics can be determined using a mass transfer analysis which is summarized below. Details of the model are described elsewhere (Logan and Alldredge 1989, Logan and Dettmer 1990). Assuming substrate uptake is proportional to substrate concentration, C , the rate of nutrient utilization can be calculated as:

$$\frac{-1}{N} \frac{dC}{dt} = k 4\pi a^2 (C - C_s) \quad (1)$$

where N is the cell concentration, k is a mass transfer coefficient, a is the cell radius, and C and C_s are the bulk and cell-surface substrate concentrations, respectively. According to the Michaelis-Menten description of enzyme kinetics, a molecule reversibly and repeatedly binds to an enzyme before successful product production. Since few enzymes achieve "kinetic perfection" (Stryer 1981), or operate at the limit of diffusion-controlled uptake rates, the substrate concentration at the cell surface is:

$$C_s = (1 - \alpha) C \quad (2)$$

where α , the collision efficiency, is limited to the range of $0 \leq \alpha \leq 1$. The mass transfer coefficient can be reduced in proportion to enzyme coverage as long as the fraction of the surface active in transport, f , is small (Dudukovic and Mills 1985). By defining $E_B = \alpha f$, where $0 \leq E_B \leq 1$, we obtain:

$$\frac{-1}{N} \frac{dC}{dt} = 4\pi a D \text{Sh} E_B C \quad (3)$$

where Sh is the dimensionless Sherwood number, defined as $\text{Sh} = ka/D$, and D is the substrate diffusivity.

For a spherical cell in stagnant fluid, the Sherwood number is unity. The Sherwood number has no physical limits, but high fluid velocities past the cell will ultimately damage the cell and decrease nutrient uptake. The Sherwood number correlation used in this study to predict the effect of fluid flow past cells (Logan and Alldredge 1989) was:

$$Sh_u = 1 + 45 Re^{0.57} \quad (4)$$

where $Re=au/\nu$ is the cell Reynolds number, u is the fluid velocity past the cell, and ν is the kinematic fluid viscosity. For a cell in laminar shear flow, Frankel and Acrivos (1968) proposed:

$$Sh_G = 1 + 0.26 Pe^{0.5} \quad (5)$$

where Pe is the Peclet number, defined as $Pe=a^2G/D \ll 1$, and G is the shear rate.

RESULTS

Effects of fluid flow on [3H]leucine uptake: Fluid motion is predicted to increase microbial uptake only if transport is not saturated. During our experiments, leucine transport was probably saturated in surface waters of Roosevelt Inlet since the leucine concentration was 45 nM (August 7) and K_s for leucine uptake is probably <1 nM. To reduce leucine concentrations and obtain non-saturated uptake kinetics, we aged the bacterial size fraction ($<0.8 \mu m$ filtrate) for 24 hr in the dark; in the absence of leucine input from other microorganisms, bacterial uptake reduced leucine concentrations to 1.7 nM. Leucine concentrations in particle-free seawater were near the detection limit (<1 nM), indicating that the filtered water used for the flow experiments was not contaminated.

At low leucine concentrations, fluid flow past cells increased [3H]leucine uptake by a factor of 8 compared to uptake at low fluid velocities (Fig. 1). [3H]Leucine uptake increased from $0.65 \text{ pg ml}^{-1} \text{ min}^{-1}$ at 3 m d^{-1} to a maximum of $5.4 \text{ pg ml}^{-1} \text{ min}^{-1}$ at $\sim 20 \text{ m d}^{-1}$. At higher fluid velocities, [3H]leucine uptake decreased to an average of $4.5 \text{ pg ml}^{-1} \text{ min}^{-1}$ for fluid velocities between $20\text{--}70 \text{ m d}^{-1}$. The maximum

rate of leucine uptake in flow experiments was 63% less than the average uptake of suspended cells ($14.5 \pm 0.7 \text{ pg ml}^{-1} \text{ min}^{-1}$, $\pm \text{SD}$). Reduced uptake by cells on the filter was observed in other experiments. The reasons for these decreases are discussed below.

To test our assumption that enhanced activity required unsaturated uptake kinetics, we repeated the flow experiment, but added radiolabeled leucine at a concentration expected to saturate uptake kinetics. The concentration of leucine in aged samples for this experiment was 1.1 nM, and was below detection ($<1 \text{ nM}$) in bacteria-free samples. When 10.8 nM [^3H]leucine was added to the bacteria-free sample used in the flow experiments, no increase in uptake was observed with fluid motion (Fig. 2). [^3H]Leucine uptake by suspended bacteria averaged $18.5 \pm 2.5 \text{ pg ml}^{-1} \text{ min}^{-1}$. Filter bound bacteria in flow fields ranging from 8–50 m d^{-1} had an average of $13.5 \pm 1.0 \text{ pg ml}^{-1} \text{ min}^{-1}$, or 27% less than suspended samples. This indicates that flow increases up take only when uptake is not saturated.

Effects of fluid flow on [^3H]glucose uptake: We also examined the effect of fluid motion on [^3H]glucose uptake using aged samples from the Roosevelt Inlet. We were unable to detect any increase in fluid motion on uptake (Fig. 3). [^3H]glucose uptake by suspended averaged $3.9 \pm 0.3 \text{ pg ml}^{-1} \text{ min}^{-1}$, when glucose was added at a concentration of 1 nM. At fluid velocities between 8–48 m d^{-1} , glucose uptake was an average of $3.1 \pm 0.4 \text{ pg ml}^{-1} \text{ min}^{-1}$, or about 23% lower than suspended samples.

Effect of fluid shear on [^3H]leucine uptake: Fig. 4 shows the ratio of leucine uptake by suspended cells to uptake by cells suspended in a homogeneous laminar shear field. At shear rates of 0–2.1 s^{-1} , fluid shear did not appreciably affect leucine uptake of either fresh or aged samples by more than $\pm 10\%$. These results are consistent with mass transfer calculations using Eq. 3 and 5. At a shear rate of 2 s^{-1} , the Sherwood number is 1.014 for a cell 0.4 μm in radius. The data shown in Fig. 4 are not sufficiently precise to detect a 1.4% increase in leucine uptake, and indicate that leucine uptake may actually have decreased by up to 10% in sheared samples.

Arsenate inhibition: To determine whether the uptake systems for leucine and glucose were similar, we examined the effect of arsenate on leucine and glucose uptake by suspended bacteria. Arsenate inhibits ATPase which hydrolyzes ATP to provide energy for some transport systems (Ames 1986). Arsenate (10 mM) completely inhibited [^3H]leucine transport over 5 min incubations (Fig 5a). In contrast, uptake of [^3H]glucose was inhibited by only 50% (Fig 5b). These results suggest that the mechanism for leucine and glucose uptake differ.

DISCUSSION

Fluid motion can substantially increase uptake by microorganisms, but this effect is a function of several factors, including the substrate type and its concentration relative to uptake kinetics. Marine bacteria held on filters in a flow stream of 10 m d^{-1} assimilated leucine 8 times faster than bacteria under similar conditions in the absence of fluid motion. This enhancement was only observed when leucine uptake was not saturated. In order for fluid motion to increase uptake, concentration gradients must be present at the cell surface. At high leucine concentrations ($\sim 11 \text{ nM}$), fluid motion had no effect on leucine uptake probably because of the lack of a concentration boundary layer.

The observed 8-fold increase in [^3H]leucine uptake at velocities between $1\text{--}20 \text{ m d}^{-1}$ is much larger than expected from the mass transfer analysis using Eq. 4. At a velocity of 20 m d^{-1} , the Sherwood number for a cell $0.4 \text{ }\mu\text{m}$ in radius is only 20% larger than for a suspended cell, indicating that fluid flow should only increase uptake by a factor of 1.2. The 8-fold increase is not only larger than predicted by a mass transfer analysis, it is also inconsistent with other experimental results. Pure cultures of bacteria (*Zoogloea ramigera*) fixed in a flow field of 1 mm s^{-1} increased by a factor of 1.55–1.65 compared to suspended cultures (Logan and Dettmer 1990). Canelli and Fuhs (1976) observed a 2.6-fold increase in phosphorus uptake by the diatom *Thalassiosira fluviatilis* ($7 \text{ }\mu\text{m}$ radius) held on a filter surface in a velocity field of 35 m d^{-1} . Since the Sherwood number is proportional to the cell radius, we expected the magnitude of

the increase in uptake by bacteria would be smaller, not larger, than increases observed for diatoms by Canelli and Fuhs.

Bacterial uptake rates of leucine and glucose by suspended bacteria were consistently larger than uptake rates determined for filter-bound bacteria. In other experiments using pure cultures of microbes (Logan and Dettmer 1990), there was no difference between uptake rates of suspended cells and filter-bound cells held in fluid flow fields at low velocities. It is likely that natural bacteria, which are smaller than pure cultured bacteria previously examined in laboratory experiments, became partially enmeshed in the filter material, reducing the exposed surface area of the cell. In the flow experiments, uptake rates at higher fluid velocities ($20\text{--}70\text{ m d}^{-1}$) averaged $\sim 65\%$ less than suspended experiments. This indicates that as much as 65% of the surface area of the cell used for leucine uptake was covered by the filter during flow experiments. Reduced cell surface area by the filter, therefore, could explain the observed reduction in leucine uptake by cells held on filters.

The area covered by outer membrane proteins involved in leucine transport (leucine porters) is important for examining the impact of flow on leucine utilization and for understanding how these bacteria survive in the low organic concentrations of seawater. This surface area can be calculated using Eq. 3. For $5.3 \times 10^6\text{ cell ml}^{-1}$, and assuming a leucine diffusivity of $7 \times 10^{-6}\text{ cm}^2\text{ s}^{-1}$ (Logan and Dettmer 1990) and a cell radius of $0.4\text{ }\mu\text{m}$, E_B equals 0.09 for an uptake rate of $14.5\text{ pg ml}^{-1}\text{ min}^{-1}$ at a leucine concentration of 1.1 nM . If we assume the porter is 100% efficient ($\alpha=1$), this E_B indicates that 9% of the surface area of free-living cells is covered by leucine porters. This percent coverage is close to the surface area of porins, which are abundant nonspecific pores or channels found in Gram-negative bacteria (e.g. *E. coli*). Assuming that each *E. coli* cell contains approximately 10^5 porins with a surface area of $0.9\text{ }\mu\text{m}^2$ (Nikaido and Vaara 1987) and a total cell surface area of $6.7\text{ }\mu\text{m}^2$, approximately 13% of the cell surface is occupied by porins. The surface area of specific leucine porters in *E.*

coli is probably much less than for marine bacteria, although we are unaware of any published estimates.

We did not observe any effect of fluid motion on uptake of glucose uptake in either new or aged water samples. Since glucose concentrations were not measured, we cannot rule out the possibility that glucose uptake was saturated and that flow actually affects glucose uptake like leucine. However, saturation of glucose uptake seems unlikely, especially in aged samples since concentrations of labile compounds like amino acids are substantially reduced in 24 h in the bacterial size fraction. Alternatively, we hypothesize that the different effects of flow on uptake of glucose and leucine is due to different transport mechanisms. The arsenate experiment provides some support for this argument. Amino acid transport is often mediated by periplasmic binding proteins (Furlong 1987) fueled by ATP hydrolysis (Ames 1986), and thus dependent on ATPase. Arsenate, a ATPase inhibitor, should block transport of compounds such as leucine (Ames 1986). That arsenate did not completely inhibit glucose uptake suggests that a binding protein is not involved in glucose uptake. Other mechanisms for glucose transport do not involve a specific outer membrane protein (e.g., the PTS system; Meadows et al. 1987, Delong and Yayanos 1987). According to these mechanisms, glucose enters the periplasmic space of Gram-negative bacteria (the most common bacteria in marine systems) via a nonspecific porin. Passive diffusion through porins requires a concentration gradient across the membrane. This gradient, and not a concentration boundary layer outside the cell, could provide the largest resistance to mass transfer into the cell. Thus, flow could enhance uptake mediated by specific binding proteins by reducing the concentration boundary layers surrounding a cell, but not uptake where flux is limited by a gradient across the outer cell membrane.

Our uptake experiments conducted on marine bacteria at shear rates below 2 s^{-1} indicate that fluid shear is ineffective at increasing microbial uptake of small molecular weight compounds such as leucine. Since free-living marine bacteria are much smaller than the Kolmogorov microscale of turbulence, fluid shear is ineffective in reducing

concentration gradients at the cell surface. Our observations are supported by mass transfer correlations (Eq. 5), which predict a maximum increase of 1.4% at shear rates below 2 s^{-1} and are consistent with previous theoretical predictions (Purcell 1978, Logan and Hunt 1987).

In conclusion, we demonstrated that fluid flow of only 20 m d^{-1} may be sufficient to cause a several-fold increase in uptake rates of selected compounds by attached bacteria. Since sinking velocities of particles may reach 200 m d^{-1} (Alldredge and Gotschalk 1988), attached bacteria may have a selective advantage over free-living bacteria in oligotrophic environments. Increased mass transfer to attached cells may partially explain why uptake by attached bacteria in natural environments is higher than that of free-living bacteria.

Acknowledgements: Funding was provided by ONR Grant N00014-88-K-0387 to B.E. Logan and N00014-87-K-0108 to D.L. Kirchman. Preliminary experiments were performed on board the R.V. Point Sur in April 1989; ship time was provided through NSF Grant OCE-88-00396 to A.L. Alldredge. The authors are grateful to J. Moss and D.B. Wilkinson for their assistance with laboratory experiments.

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Fig. 1. The effect of fluid velocity on leucine uptake by bacteria (aged samples, 1 nM [^3H]leucine added).

Fig. 2. Leucine uptake by bacteria fixed in a flow field (aged samples, 10.8 nM of [^3H]leucine added).

Fig. 3. Glucose uptake by bacteria (aged samples, 1 nM [^3H]glucose added).

Fig. 4. The ratio of [^3H]leucine (1 nM) uptake by bacteria suspended in a laminar shear field to uptake by cells in undisturbed fluid. Fresh (\blacktriangle) and aged (\blacksquare) samples.

Fig. 5. The effect of arsenate (10 nM) addition on bacterial uptake of (a) [^3H]leucine and (b) [^3H]glucose.

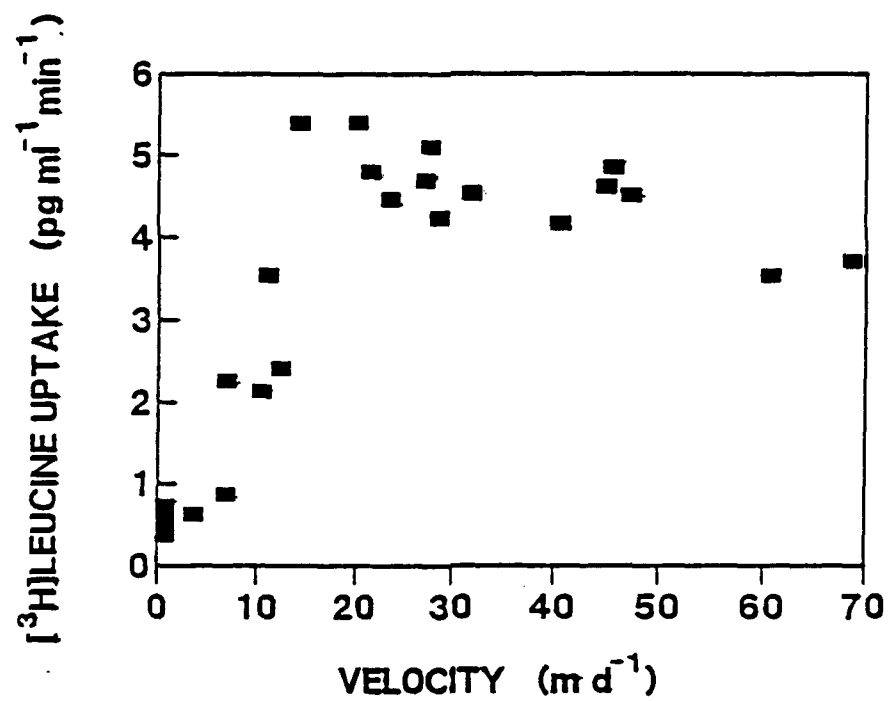


Fig. 1. The effect of fluid velocity on leucine uptake by bacteria (aged samples, 1 nM [³H]leucine added).

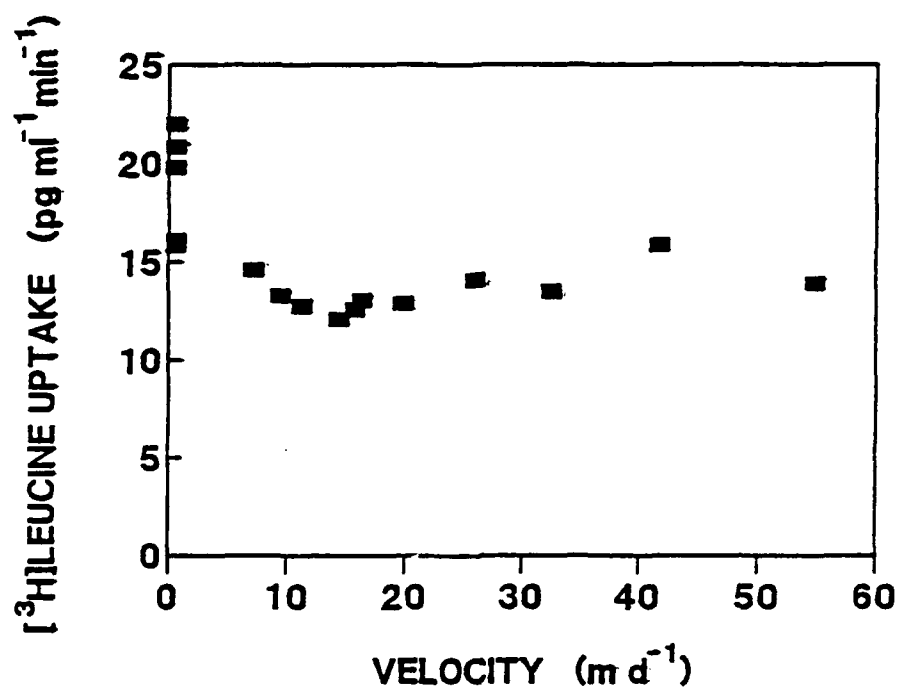


Fig. 2. Leucine uptake by bacteria fixed in a flow field (aged samples, 10.8 nM of [³H]leucine added).

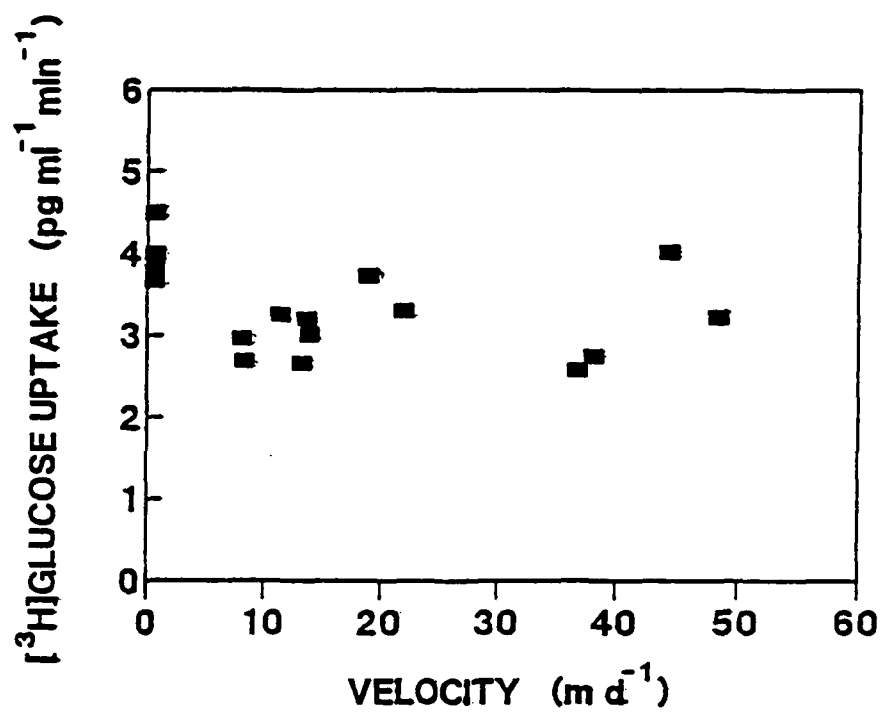


Fig. 3. Glucose uptake by bacteria (aged samples, 1 nM $[^3\text{H}]$ glucose added).

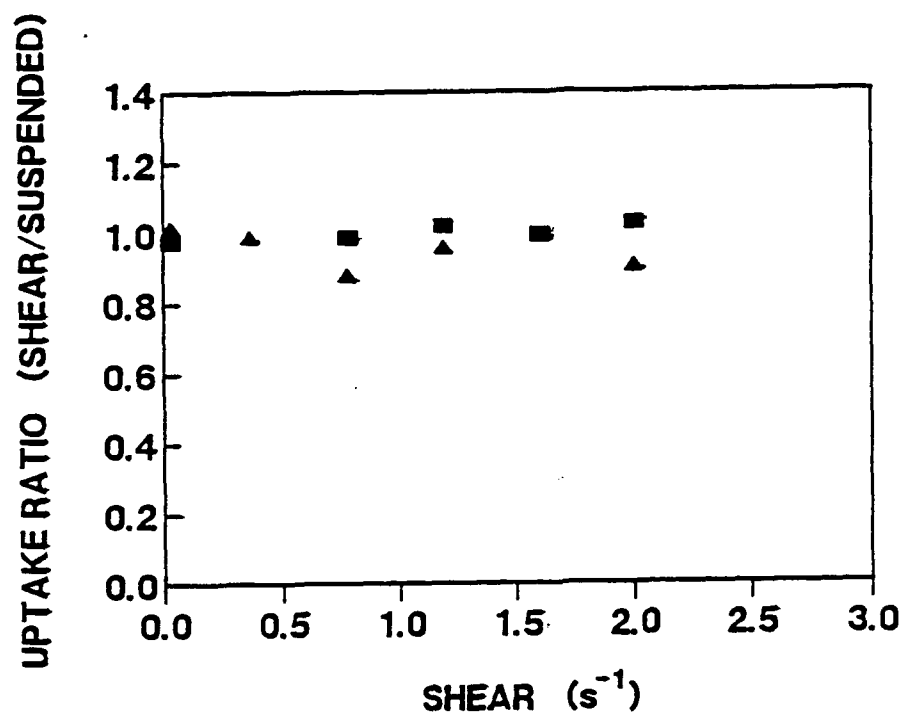


Fig. 4. The ratio of [³H]leucine (1 nM) uptake by bacteria suspended in a laminar shear field to uptake by cells in undisturbed fluid. Fresh (▲) and aged (■) samples.

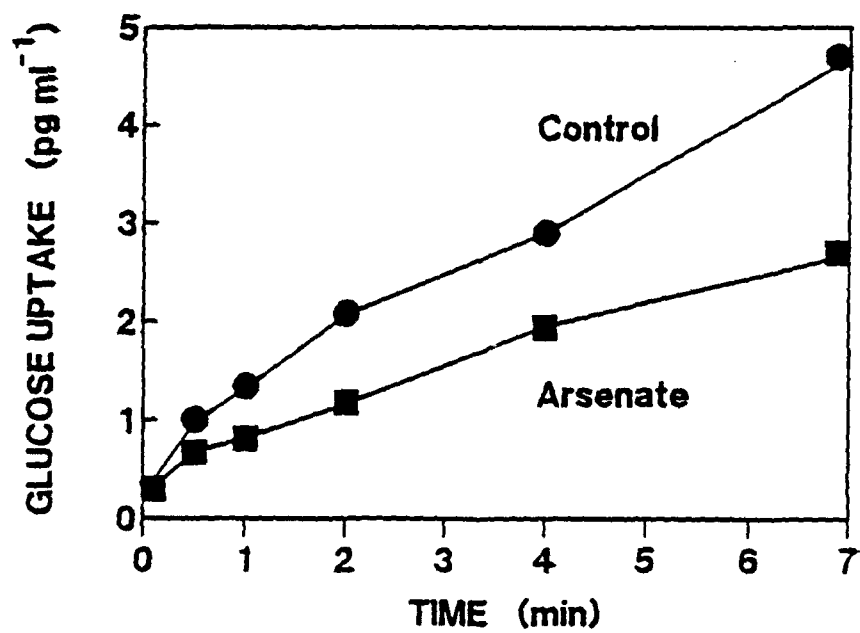
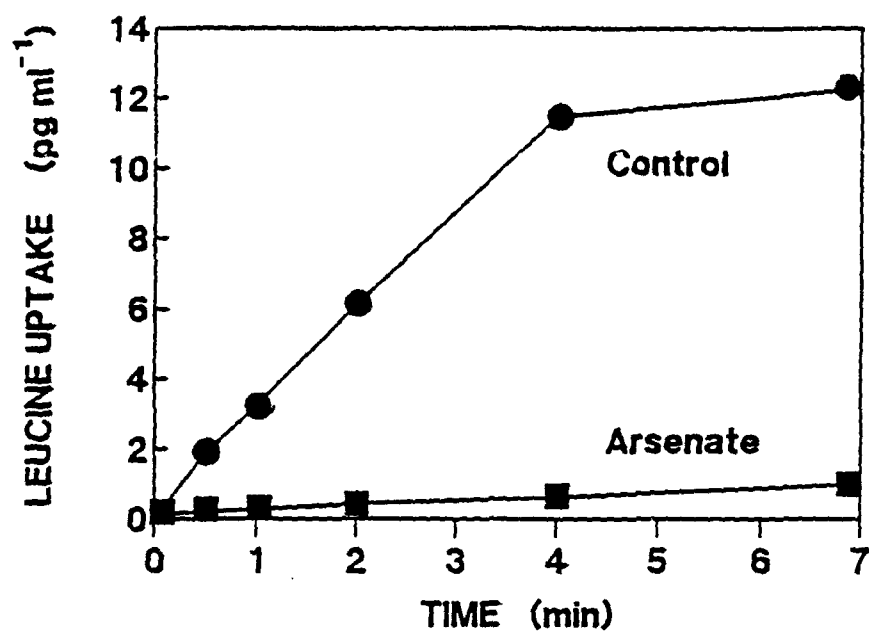


Fig. 5. The effect of arsenate (10 nM) addition on bacterial uptake of (a) [^3H]leucine and (b) [^3H]glucose.

Potential for increased nutrient uptake by flocculating diatoms

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Abstract

Blooms of chain-forming diatoms commonly flocculate into centimeter-sized aggregates of living, vegetative cells following nutrient depletion in surface waters off southern California. We examined the hypothesis that diatom cells within aggregates experience increased nutrient uptake relative to unattached cells. We measured in situ settling velocities of 49 to 190 m d⁻¹ and calculated porosities of 0.99931 to 0.99984 ($\pm < 0.03\%$) for 12, newly-formed diatom flocs ranging from 0.19 to 4.2 cm³ in volume and 7 to 22 mm in equivalent spherical diameter. Using permeability–porosity relationships, we calculated intra-aggregate flow velocities of 20 to 160 $\mu\text{m s}^{-1}$. Although subject to considerable uncertainty, a Relative Uptake Factor analysis based on mass transfer equations suggests that diatoms fixed within aggregates undergoing gravitational settling can take up nutrients up to 2.1 ± 0.4 times faster than unattached diatoms experiencing laminar shear. Increased nutrient uptake by aggregated diatoms may be important in understanding the reasons for diatom floc formation.

Introduction

Blooms of chain-forming diatoms often disappear rapidly from surface ocean waters following nutrient depletion (Guillard and Kilham 1977, Smetacek 1985). This mass sedimentation occurs via flocculation of chains into loose, centimeter-sized aggregates of living, vegetative cells (Alldredge and Silver 1988, Kranck and Milligan 1988) which sink at rates of 50 to 200 m d⁻¹, one to two orders of magnitude higher than unaggregated chains (Alldredge and Gotschalk in press). Mass sinking of diatoms has been equated with mass mortality (Walsh 1983), and, at first glance, mechanisms resulting in the rapid settlement of viable cells out of the euphotic zone appear maladaptive. However, Smetacek (1985) suggested that mass flocculation removes diatoms from warm, nutrient-depleted surface waters, where their viability is low into dark, colder waters where they may

remain viable for long periods of time as resting spores and vegetative resting stages. Later resuspension of these resting stages back into the euphotic zone would reseed surface waters with diatoms when conditions were again favorable for population growth.

While this evolutionary explanation of diatom flocculation is supported by existing literature on diatom biology, it does not preclude a more immediate adaptive advantage for mass flocculation. There are several possible immediate advantages for cell growth within the microhabitat of a porous aggregate, including interactions between adjacent microorganisms, (i.e., commensalism, mutualism, and exchange of genetic material) and potential protection from certain predators. The most important advantage in highly porous aggregates, however, may be increased nutrient uptake (Logan and Hunt 1987). Many types of cells, including yeasts and bacteria, aggregate when nutrients are stressed (see Calleja 1984) and diatom flocculation in nature also appears to be triggered by nutrient limitation (Smetacek 1985). Moreover, cultures of *Chaetoceros debilis*, a representative of the most common genus in natural diatom flocs, flocculate under gentle rotation in the laboratory only when they reach stationary growth phase, (i.e., nutrient limitation) and not before (Alldredge unpublished data). Logan and Hunt (1987) predicted that advective flow through porous bacterial aggregates would increase the overall uptake of large molecular weight, dissolved organic molecules by attached, relative to unattached, bacteria. Moreover, Munk and Riley (1952) suggested that phytoplankton cells experiencing faster sinking velocities also have accelerated nutrient absorption and increased growth rates despite decreased concentrations of nutrients. Although Munk and Riley (1952) did not consider the case of phytoplankton in aggregates, recent experiments on the absorption of a large molecular weight dye by different types of marine snow support the existence of bulk fluid motion through natural marine aggregates (Logan 1987) and suggests that diatoms in sinking aggregates experience relatively more rapid fluid flow than unattached diatoms.

In this study we examined the hypothesis that diatom cells within aggregates experience increased overall transport of nutrients relative to unattached cells, i.e., that an immediate adaptive advantage of diatom flocculation is increased nutrient uptake by the aggregated cells. We used measured sinking velocities and porosities to calculate intra-aggregate flow velocities past cells fixed within natural diatom flocs. We then used mass transfer theory to predict the overall nutrient uptake of cells within natural diatom flocs relative to unattached cells.

Methods

Experimental

The settling velocities of 12 undisturbed, newly-formed diatom flocs were measured directly in situ by SCUBA divers in surface waters of the Santa Barbara Channel, California (34°23'N, 119°50'W) in July 1986. Settling velocities were determined, as described in Alldredge and Gotschalk (1988), by measuring the time required for each aggregate to sink to a spot of neutrally buoyant fluorescein dye placed 3 cm below the aggregate in the water column. Aggregate volume was determined from photographs taken in situ and each aggregate was collected for gravimetric determination of dry weight.

Porosity, p , was calculated directly from measurements of aggregate volume and dry weight using the equation

$$p = 1 - \frac{W}{P\bar{V}} \quad (1)$$

where W is the dry weight of the aggregate, \bar{V} is aggregate volume and P is the density of the diatoms composing the aggregate, here assumed to be 1.1047 g cm^{-3} . This value was calculated from ambient seawater density (1.0247 g cm^{-3} , Alldredge and Gotschalk 1988) assuming an excess density for nutrient-depleted living diatom cells of 0.08 g cm^{-3} (Eppeley et al. 1967). Species composition and mean cell sizes of diatoms in flocs were determined by counting and measuring cells in eight additional flocs collected at the same time.

Theoretical

Measured sinking velocities were compared to settling velocities of highly porous spherical aggregates, determined from a force balance as

$$U_p^2 = \frac{8g(P - P_f)d}{6C_D P_f} \quad (2)$$

where U_p is the predicted settling velocity, g is the acceleration due to gravity, P is the particle density, P_f is the fluid density, d is the aggregate diameter, and C_D is the drag coefficient. For permeable aggregates with Reynolds numbers (Re) between 7 and 120 undergoing gravitational set-

ling, Masliyah and Polikar (1980) determined the empirical expression of the drag coefficient as:

$$C_D = \frac{24\Omega}{Re} [1 + 0.0853 Re^{(1.093 - 0.105w)}] \quad (3)$$

where $Re = U_p d / \nu$, ν is the kinematic viscosity of the fluid, $w = \log_{10}(Re)$, and Ω is determined from

$$\Omega = \frac{2\xi^2 [1 - \tanh \xi \xi]}{2\xi^2 + 3[1 - \tanh \xi \xi]} \quad (4)$$

where ξ is the dimensionless size of the aggregate, defined as $\xi = a_f K^{-1/2}$, a_f is the aggregate radius, and K is the permeability of the aggregate.

The permeability of highly-porous aggregates was defined as a function of the aggregate structure and porosity using (Davies 1952):

$$K^{-1} = 16 a_c^{-2} \phi^{1.5} (1 + 56 \phi^3), \quad (5)$$

where a_c is the cell radius, and ϕ is the occupied volume fraction of the aggregate, equal to the quantity $(1 - p)$. We used the Davies correlation since this expression was used by Masliyah and Polikar (1980) in settling experiments to validate the existence of flow through permeable aggregates with $0.2 < Re < 120$.

The average intra-aggregate velocity, u , through a permeable spherical aggregate undergoing gravitational settling (Adler 1981) is:

$$u = U \frac{a_f^*(\xi)}{a_f} \quad (6)$$

where U is the measured in situ sinking velocity, and a_f^* is a function of the dimensionless variable ξ contained in Adler (1981). When a highly porous aggregate is permeable, streamlines cross the surface of the aggregate, and advect fluid past diatoms fixed within the interior of the aggregate. Therefore, a diatom within a rapidly sinking permeable aggregate potentially experiences higher fluid flow past its surface than does an unattached cell, which either sinks very slowly or moves entirely with the bulk fluid.

The rate of nutrient absorption by a cell is a function of nutrient concentration and flow field: fluid flow past a cell increases the rate of nutrient absorption by the cell (Munk and Riley 1952). Following the analysis of Logan and Hunt (1987), we incorporated this hydrodynamic effect into a first-order kinetic model using a mass transfer coefficient to increase cell kinetics with increased fluid flow past the surface of the cell. Since attached cells within aggregates are exposed to lower nutrient concentrations than unattached cells, nutrient removal by all cells within the aggregate was compared to nutrient removal by an equivalent number of cells freely dispersed in the bulk fluid using a Relative Uptake Factor, as described below.

The rate of mass transfer to a single cell, P_c , in any fluid environment (Logan and Hunt 1987) is:

$$P_c = 4\pi a_c D E_B \text{Sh } C \quad (7)$$

where D is the nutrient diffusivity, assumed to be $10^{-5} \text{ cm}^2 \text{ s}^{-1}$, and C is the nutrient concentration. The Sherwood number is a dimensionless ratio of the rate of mass transfer by advection to the rate of mass transfer by diffusion, and is defined here as $Sh = k a_c D$, where k is the mass transfer coefficient. For a spherical object of radius a_c , the Sherwood number is unity for mass transfer by diffusion alone, and greater than unity for increased mass transfer due to fluid motion around the cell. The kinetics of nutrient uptake are incorporated in the transport model using a collector efficiency, E_B , which defines uptake as that fraction of collisions between substrate and cells that are successful; for instantaneous reactions, $E_B = 1$, and in the absence of nutrient uptake, $E_B = 0$. The mass transfer calculations presented below use $E_B = 0.025$, as determined by Logan (1986) from experimental data reported by Canelli and Fuhs (1976).

In order to examine mass transfer to attached and unattached diatoms, two Sherwood numbers are required: one for diatoms fixed in a flow field, and a second for unattached diatoms experiencing laminar shear. Sherwood numbers for diatoms within a permeable aggregate, or for unattached diatoms sinking in undisturbed fluid, were derived from data reported by Canelli and Fuhs (1976) on phosphorus uptake by cells of *Thalassiosira fluviatilis* fixed in a flow field. Using their data, we empirically determined the following correlation for the Sherwood number as a function of the Reynolds number:

$$Sh = 1 + 45 Re^{0.57} \quad (8)$$

Many mass transfer relationships indicate the Sherwood number is proportional to the Reynolds number to the 0.5 power, and our correlation is in good agreement with this value, since the standard error of the power in Eq. 8 is 0.08. In Fig. 1 we compare the phosphorus flux data reported by Canelli and Fuhs (their experiments 2 and 3, in Fig. 2) with fluxes predicted using the above correlation (Eq. 8). At lower concentrations of phosphorus ($10 \mu\text{g l}^{-1}$), flux values are less than $10^{-11} \mu\text{g-P } \mu\text{m}^{-2} \text{ min}^{-1}$, and the observed and predicted fluxes are in relatively close agreement. However, at larger phosphorus concentrations ($100 \mu\text{g l}^{-1}$), fluxes are

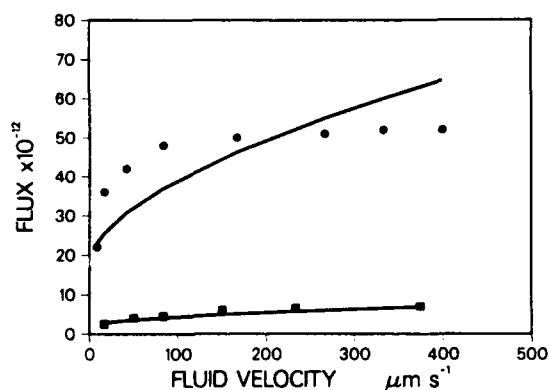


Fig. 1. Phosphorus fluxes ($10^{-12} \mu\text{g-P } \mu\text{m}^{-2} \text{ min}^{-1}$) calculated using Eq. 8 (continuous line) versus data reported by Canelli and Fuhs (1976): ■ – their experiment 2 ($a_c = 7.3 \mu\text{m}$, $C = 10 \mu\text{g-P l}^{-1}$); ● – their experiment 3 ($a_c = 8.6 \mu\text{m}$, $C = 100 \mu\text{g-P l}^{-1}$)

underestimated for fluid velocities less than $200 \mu\text{m s}^{-1}$, and overestimated for velocities greater than $200 \mu\text{m s}^{-1}$. Canelli and Fuhs' data suggest that a saturation value was reached in their experiments.

The fluid environment of an unattached diatom can be characterized as laminar shear, since diatoms freely suspended in the ocean are much smaller than the Kolmogorov microscale of turbulence. The Sherwood number for a sphere in laminar shear flow, Sh_G , (Frankel and Acrivos 1968) is:

$$Sh_G = 1 + 0.26 Pe_G^{1/2} \quad (9)$$

where Pe is the dimensionless Peclet number, defined as $Pe_G = a_c^2 G D$, and G is the fluid shear rate. Eq. 9 is valid for Peclet numbers much less than unity, and is only used for calculations of the rate of mass transfer to unattached diatoms.

The ratio of the overall nutrient uptake by aggregated cells to the nutrient uptake by an equivalent number of unattached cells is defined as the Relative Uptake Factor (Logan and Hunt 1987). For nutrient uptake by cells in a permeable aggregate undergoing gravitational settling, compared to unattached cells suspended in laminar shear flow, the Relative Uptake Factor, v_a , is:

$$v_a = \frac{Sh_u [1 - \exp(-\phi_a)]}{Sh_G \phi_a} \quad (10)$$

where ϕ_a , the dimensionless advective Thiele Modulus, is

$$\phi_a = \frac{2 a_f k_u}{u} \quad (11)$$

and a_f is the aggregate radius, and k_u is the first order uptake rate constant describing nutrient removal within the aggregate. From the rate of uptake per cell (Eq. 7), k_u is

$$k_u = \frac{3 E_B Sh_u D (1 - p)}{a_c^2} \quad (12)$$

The ratio of Sherwood numbers in Eq. 10 accounts for the different fluid mechanical environment of the unattached cells (laminar shear) and the cells within the aggregate (fixed in a flow field). The Thiele modulus, as defined in Eq. 11, is a ratio of the rate of mass transfer to attached diatoms, to the rate of fluid flow. Since nutrients are removed by cells as fluid flows through the aggregate, the Thiele modulus accounts for decreased nutrient concentrations in the aggregate interior.

For growth within the aggregate to be advantageous to all cells within the aggregate, the Relative Uptake Factor must be greater than unity. For $v_a < 1$, only cells near the surface of the aggregate would benefit from advective flow through the aggregate. Since first order kinetics are implied for all substrate concentrations below the bulk nutrient concentration, a Relative Uptake Factor analysis does not require that the bulk nutrient concentration be known since uptake is measured relative to unattached cells in the bulk fluid.

Results

Taxonomic composition of flocs expressed as percent of total cell number is shown in Table 1. Three species of diatoms, *Chaetoceros radicans*, *C. debilis*, and *Nitzschia* sp., comprised $85 \pm 1\%$ of the cells examined. The remaining cells were classified as centrics, pennates, or dinoflagellates. Aggregates were very loosely held together by tangled spines. Neither visual nor microscopic examination revealed the presence of abundant mucus exudates within the aggregates. The average cell volume of diatoms within the flocs was calculated based on the volumes of the three major diatom species, assuming their shapes to be cylinders. A cell with a volume equivalent to the average volume of cells within the flocs was calculated to have an average radius of $7 \mu\text{m}$. This average radius was used in subsequent calculations for diatom characteristics.

The porosity of 12 diatom flocs is shown as a function of aggregate volume in Fig. 2. Aggregate volumes ranged between 0.19 and 4.2 cm^3 ; based on these volumes, the diameter of a spherical aggregate of equal volume varied between 7 and 20 mm . Porosity increased non-linearly with aggregate volume, ranging between 0.99931 and 0.99984 . A maximum error in porosity, Δp , was determined using a first-term

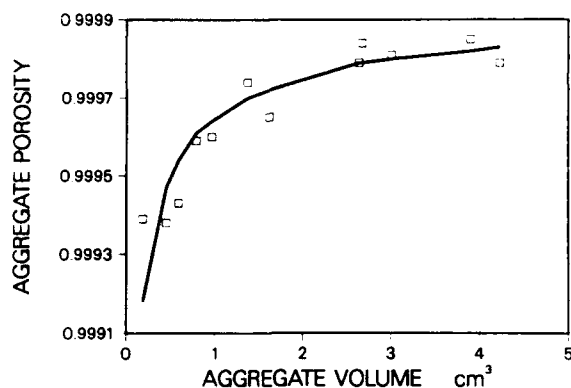


Fig. 2. Aggregate porosity (p) for 12 diatom flocs as a function of floc volume (V , cm^3), with regression line $p = 1 - 3.5 \times 10^{-4} V^{-0.50}$

Table 1. Percentage composition of diatom flocs collected from the Santa Barbara Channel, July 1986, by phytoplankton cell number. Reported percentages ignore whether cells present in chains. ($N = 10$, S.D. = standard deviation)

Species	Mean % (\pm S.D.)	Mean cell dimensions (diameter \times height, μm)	Mean cell volume (μm^3)
<i>Chaetoceros debilis</i>	18 ± 2	21×15	5 440
<i>Chaetoceros radicans</i>	58 ± 6	9×13	780
<i>Nitzschia</i> sp.	10 ± 2	7×30	1 150
Miscellaneous			
Centrics	8 ± 3	15×15	
Pennates	3 ± 1	10×30	
Dinoflagellates	4 ± 2	12×2	

Taylor Series expansion (Mickley et al. 1957) for porosity as a function of three variables, or

$$\Delta p = \left| \frac{\partial p}{\partial W} \right| \Delta W + \left| \frac{\partial p}{\partial V} \right| \Delta V + \left| \frac{\partial p}{\partial P} \right| \Delta P. \quad (13)$$

Using Eq. 1, this expression becomes:

$$\Delta p = \frac{1}{pV} \Delta W + \frac{W}{V^2} \Delta V + \frac{W}{V^2 P} \Delta P. \quad (14)$$

To calculate Δp , we used measured values, and assumed the following maximum errors based on data in Alldredge and Gotschalk (1988): $\Delta W = \pm 10\%$, $\Delta P = \pm 0.08 \text{ g cm}^{-3}$, and $\Delta V = \pm 30\%$. As shown in Table 1, the errors in porosity are small and are $< 0.03\%$.

Measured settling velocities of diatom flocs in situ ranged between 49 and 190 m d^{-1} , with an average of $110 \pm 40 \text{ m d}^{-1}$ (Fig. 3). We also calculated the settling velocities using Eqs. 1 to 4. Predicted settling velocities of the 12 diatom flocs ranged between 73 and 120 m d^{-1} and were not statistically different (Students' t -test) from the observed settling velocities of these flocs, even though the calculated velocities did not appreciably increase with aggregate diameter. The average predicted settling velocity of $100 \pm 12 \text{ m d}^{-1}$ does not differ significantly from the in situ settling velocity of 110 m d^{-1} . Similar values for the observed and predicted settling velocities support the calculation of an equivalent aggregate radius used in this analysis, and the application of the permeable aggregate model for diatom flocs.

Measured sinking velocities were used in Eqs. 5 and 6 to calculate intra-aggregate flow velocities between 24 and $160 \mu\text{m s}^{-1}$ (2 to 14 m d^{-1}), with an average intra-aggregate velocity of $70 \mu\text{m s}^{-1}$ (6 m d^{-1}), or about 5% of the observed aggregate settling velocity for 12 diatom flocs (Table 2). A wide range of intra-aggregate velocities of 60 to $160 \mu\text{m s}^{-1}$ are predicted for aggregates in a narrow size range of 17 to 20 mm in diameter. This reflects the variable porosity, and therefore, permeability, of diatom flocs.

The overall rate of nutrient uptake by aggregated diatoms undergoing gravitational settling was compared to: (1) unattached cells in laminar shear flow and (2) unattached cells sinking in an undisturbed fluid, using a Relative Uptake Factor analysis. Fluid shear rates within the ocean mixed layer based on turbulent energy dissipation rates are highly variable, with ranges of 0.15 to 0.44 s^{-1} (Moum and Caldwell 1985), 0.001 to 1 s^{-1} (Shay and Gregg 1984), and 0.01 to 0.04 s^{-1} (Oakey and Elliott 1982). The larger the shear rate, the greater the uptake of an unattached cell and the smaller the Relative Uptake Factor. In the first case, we assumed the largest fluid shear rate of 1 s^{-1} as an upper limit of fluid shear for unattached cells. This results in a conservative evaluation of the benefits of diatom nutrient uptake within a settling aggregate. For the 12 diatom flocs, we calculated Relative Uptake Factors between 1.0 and 2.1 (average of 1.4), indicating that diatoms within the aggregate could potentially utilize dissolved nutrients up to 2.1 times faster than unattached diatoms experiencing fluid shear in

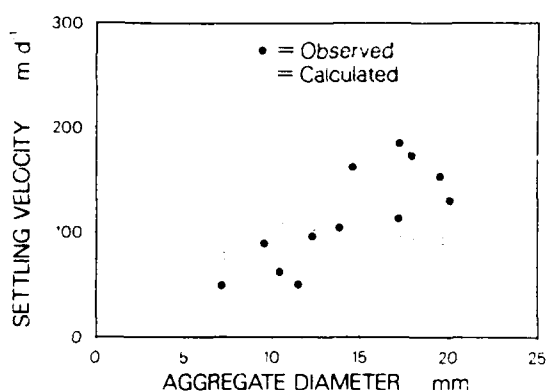


Fig. 3. Observed and calculated settling velocities of diatom flocs

Table 2. Properties of diatom flocs and predicted intra-aggregate velocities and relative uptake factors

Floc diam. (mm)	Floc porosity	Measured settling velocity (m d ⁻¹)	Intra-aggregate velocity (μm s ⁻¹)	Relative uptake factor
7.1	0.99939 ± 0.00029	49	35	1.3 ± 0.3
9.5	0.99938 ± 0.00029	89	36	1.2 ± 0.4
10.0	0.99943 ± 0.00027	62	24	1.0 ± 0.4
12.0	0.99959 ± 0.00019	50	26	1.1 ± 0.3
12.0	0.99960 ± 0.00019	96	45	1.3 ± 0.3
14.0	0.99974 ± 0.00012	100	71	1.5 ± 0.3
15.0	0.99965 ± 0.00017	160	67	1.5 ± 0.4
17.0	0.99979 ± 0.00010	110	70	1.6 ± 0.3
17.0	0.99984 ± 0.00008	190	160	2.1 ± 0.4
18.0	0.99981 ± 0.00009	170	110	1.8 ± 0.4
20.0	0.99985 ± 0.00007	150	120	1.9 ± 0.4
20.0	0.99979 ± 0.00010	130	60	1.5 ± 0.3

the water column (Table 2). This analysis accounts for nutrient depletion within the aggregate, and requires that both unattached diatoms and the diatom floc are exposed to low nutrient concentrations where a first order uptake model accurately describes nutrient uptake kinetics.

In case 2, Relative Uptake Factors were also calculated assuming unattached cells sank in undisturbed fluid. Smayda and Boleyn (1967) found that the mean sinking rate of chains of *Chaetoceros lauderi* up to 20 cells long ranged from 0.46 to 1.54 m d⁻¹, with no clear relationship between sinking speed and culture age. This species had a cell diameter ranging from 11 to 28 μm making it similar in size to the two *Chaetoceros* species that made up an average of 76% of the diatoms in our flocs. Assuming a sinking velocity of 1 m d⁻¹ for unattached cells, the Relative Uptake Factors of cells within aggregates ranged between 1.1 and 2.2. This suggests that consideration of unattached cells as either sinking or suspended within a laminar shear field is unimportant in obtaining Relative Uptake Factors greater than unity for diatom flocs.

The calculation of a Relative Uptake Factor is based on a series of measured values and equations. The maximum error in this calculation can be estimated using a Taylor

series expansion about Eq. 10, by calculating errors in ϕ , using Eqs. 11 and 12, and Sh_a , using Eq. 8. An error for Sh_a was not determined since an upper limit of G was used in the calculation of this Sherwood number. The error in ϕ was determined for constant E_B , D , a , and r , with the following assumed errors: $\Delta W = \pm 10\%$, $\Delta P = \pm 0.08 \text{ g cm}^{-3}$, $\Delta V_f = \pm 30\%$, and $\Delta u = \pm 50\%$. These maximum errors are shown in Table 2 for each Relative Uptake Factor. On the average, $\gamma = 1.4 \pm 0.3$, which is larger than unity. Within these large error bounds, four Relative Uptake Factors were less than unity, indicating that within some aggregates, all cells may not have increased nutrient uptake compared to unattached cells.

Discussion

The porosities of diatom flocs were highly variable, but were in excess of 0.99910 for the 12 diatom flocs analyzed. These values are higher than average porosities estimated for aggregates found in engineered bioreactors. For example, porosities for activated sludge flocs range from 0.79 to 0.90, (Mueller et al. 1966), and porosities for mold pellets range from 0.800 to 0.988 (Yano et al. 1961). Since the size of an aggregate is a function of fluid shear (Hunt 1986), the high diatom floc porosities are reasonable for aggregates formed in low shear environments typical of natural systems. In engineered systems, such as wastewater treatment systems, shear rates are in the range of 100 to 1000 s⁻¹. Large shear rates contribute to aggregate breakup, re-compaction, and the formation of smaller aggregates with reduced porosities. Porosities of diatom flocs are similar to aggregate porosities determined using collision calculations based on random particle trajectories in computer models (Tambo and Watanabe 1979). These computer-generated aggregates, described as fractals (Witten and Cates 1986), typically neglect aggregate breakup.

The Relative Uptake Factors for the 12 diatom flocs were determined from several aggregate characteristics, and a series of relationships drawn from the literature, to be in the range of 1.0 ± 0.3 to 2.1 ± 0.4 (average 1.4 ± 0.3). This indicates flocculation can increase nutrient uptake by attached diatoms. In our error analysis, we estimated the cumulative effect of errors in measured parameters still resulted in Relative Uptake Factors greater than unity. Two equations were not included in the error analysis: Eq. 5, used to relate aggregate porosity and permeability, and Eq. 6, used to determine the intra-aggregate velocity. Several correlations have been found to provide good agreement between experimentally determined permeabilities and porosities for fibrous media at $Re < 10$ (Jackson and James 1986). However, we are unaware of correlations for high-porosity aggregates at the slightly larger Reynolds numbers ($3 \leq Re \leq 31$) determined for settling diatom flocs. As previously stated, we used the Davies correlation since this expression was supported by experiments conducted by Masilyah and Polikar (1980) for $0.2 < Re < 120$. Instead of performing an error analysis on Eqs. 5 and 6, we calculated

the error in the Relative Uptake Factor by assuming the intra-aggregate velocity obtained from these equations was only accurate within $\pm 50\%$. Even with this large variation in intra-aggregate velocity, only four of the Relative Uptake Factors, within a standard error, were less than unity. Therefore, while our hypothesis remains physically untested, our calculations provide strong evidence that diatom flocculation can increase nutrient uptake.

There are two defined limits to a Relative Uptake Factor analysis of mass transfer to attached diatoms. The lower limit results if the advective flow of nutrients into the aggregate is less than the diffusive flux. This occurs when the Peclet number for the floc, $Pe_f = a_f u D \ll 1$ (Logan and Hunt 1988). For the diatom flocs in this study, this would occur for $u \ll 0.01 \text{ m d}^{-1}$, a velocity much lower than calculated values (Table 2). However, since unattached cells settle at $\sim 1 \text{ m d}^{-1}$, intra-aggregate flows would have to be greater than 1 m d^{-1} to confer any additional nutrient advantage to attached diatoms.

The upper limit of the Relative Uptake factor is defined by the ratio of Sherwood numbers in Eq. 10. As the intra-aggregate velocity increases, and the aggregate porosity decreases, the ratio $[1 - e^{-\phi}] \phi$ approaches unity. Therefore, the overall uptake by attached cells cannot exceed the uptake by a cell fixed in a flow field of velocity u , divided by the uptake of a cell in laminar shear (or sinking at 1 m d^{-1}). However, this ratio of Sherwood numbers may still underestimate uptake by attached cells since the high fluid velocities at an aggregate surface are not included in our calculations. We assumed the floc was homogeneous and spherical in shape, and that all cells within the aggregate resided in a fluid field described by the average intra-aggregate fluid velocity. This oversimplification of the structure and shape of a marine snow aggregate was necessary to obtain an analytical solution. Natural aggregates often have long comet-shaped tails and irregular shapes; the exact shape of the aggregate, however, is not expected to be important in mass transfer calculations (Aris 1975). More important to our calculations is the assumption of the homogeneity of the aggregate. Many aggregates contain internal voids (see Alldredge and Silver 1988, Fig. 2a, and Alldredge and Gotschalk 1988, Fig. 1b for visual examples). As a result, many cells (more than for a sphere) will be at the aggregate surface and will experience fluid velocities much larger than the average intra-aggregate velocity calculated for the homogeneous spherical aggregates in this study. These surface cells could remove nutrients much faster than cells within the aggregate interior. This effect would increase the benefits of attachment.

The maximum benefit of attachment for diatoms in permeable aggregates, for any model, is limited by the maximum possible increase in uptake with respect to fluid hydrodynamics. This hydrodynamic effect is described mathematically in terms of Sherwood number correlations for cells in laminar fluid shear and for spheres fixed in a fluid field. Despite work by Munk and Riley (1952) over three decades ago, little improvement has been made in quantifying the effect of hydrodynamics on nutrient uptake by phytoplank-

ton. The physics of mass transfer to spheres and other objects is well understood in the field of chemical engineering, and kinetic models relating enzyme kinetics to bulk nutrient concentration are well studied by biologists. However, the increase in uptake kinetics as a result of fluid hydrodynamics in biological systems is not well understood.

To our knowledge, only two studies on the effect of hydrodynamics on nutrient uptake by marine diatoms can be used to calculate a Sherwood number. The first study, by Canelli and Fuhs (1976), investigated phosphorus uptake by cells of *Thalassiosira fluviatilis* fixed in a flow field and is used in our calculations (Eq. 8). In Fig. 4, two other Sherwood number correlations that have been used for similar purposes in other studies are compared to Eq. 8. The first correlation, developed by Brian and Hales (Sherwood et al. 1975), and used by Logan and Hunt (1987, 1988) to describe uptake by cells fixed in a flow field is:

$$Sh_u = (1 + 0.48 Pe_u^{2/3})^{1/2}. \quad (15)$$

A second correlation was used by Munk and Riley (1952) in their classic study for sinking spheres; their correlation is:

$$Sh = 1 + 0.5 Pe + 0.60 Pe^2. \quad (16)$$

Fig. 4 shows that at velocities $\leq 160 \mu\text{s}^{-1}$ both Eqs. 15 and 16 underestimate the effect of fluid flow when compared to the Sherwood number correlation derived using the experimental data of Canelli and Fuhs. All three correlations indicate Sherwood numbers should continue to increase with fluid velocities greater than $160 \mu\text{s}^{-1}$, but this observation is not supported by Canelli and Fuhs' data (Fig. 1). These results suggest there is some effect of fluid velocity that is not adequately described in our mass transfer analysis. Since the calculated intra-aggregate velocities shown in Table 2 are less than $\leq 160 \mu\text{s}^{-1}$, the Relative Uptake Factors in Table 2 are conservative.

The second study that can be used to determine a Sherwood number correlation was reported by Pasciak and Gavis (1975, Fig. 5) on nitrate uptake by *Ditylum brightwellii*. Shown in Fig. 5 is a comparison of Sherwood numbers for a $41 \mu\text{m}$ cell (the equivalent spherical radius of *D. brightwellii*) to Sherwood numbers calculated using Eq. 9. Much greater uptake for unattached diatoms as a result of fluid shear is predicted from Eq. 9 than was observed by Pasciak and Gavis (1975). This suggests that we overestimated the uptake of unattached diatoms in our calculations, which would lead to a larger Relative Uptake ratio than shown in Table 2. Thus, experimental work by Pasciak and Gavis (1975), as well as by Canelli and Fuhs (1976), suggests that our comparison of the nutrient advantage experienced by aggregated over unattached diatoms is conservative.

Flocculation has historically been viewed as a disadvantage to attached cells for two reasons. First, aggregates have been viewed as impermeable, resulting in decreased nutrient availability to cells within the aggregate interior (Yano et al. 1961). Second, since flocculation increases settling velocity, diatoms may be removed faster from the euphotic zone than unattached cells. Smetacek (1985), for example, has argued that aggregation and mass sinking represent a transition

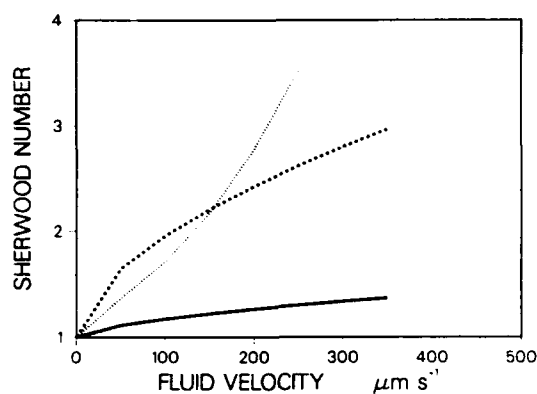


Fig. 4. Sherwood numbers of a cell 7 μm in radius fixed in a flow field, calculated using: Eq. 8, based on data in Canelli and Fuhs (1976) (dashed line); Eq. 16, the Brian and Hales Correlation (Sherwood et al. 1975) (continuous line); Eq. 15, a correlation used by Munk and Riley (1952) (dotted line)

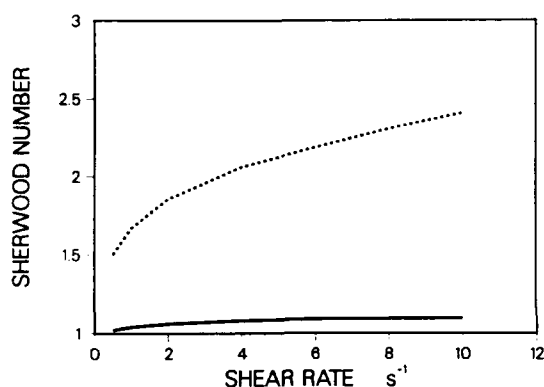


Fig. 5. Sherwood numbers calculated using Eq. 9 (dashed line) for a cell 41 μm in radius as a function of fluid shear rate versus results adapted from Pasciak and Gavis (1975) (continuous line)

from a growing to a resting stage in the life histories of diatoms. Aggregated cells sink out of nutrient-depleted surface waters into deep, nutrient-rich water and await resuspension back into the euphotic zone as resting stages. While this evolutionary explanation for the mass flocculation of diatom blooms may be correct, resting stages are rare in diatom flocs observed in surface waters (Alldredge and Gotschalk in press) although they may form at depth. Our data suggest that the flocculation of diatom blooms may be immediately adaptive because aggregation alters the fluid environment of a cell. A single unattached cell or chain is contained within a microscale eddy and must move with the bulk fluid; however, fluid flow around and through a rapidly sinking aggregate actually alters the fluid environment of associated cells, and increases nutrient uptake by attached cells compared to unattached cells. Flocculation may, therefore, be advantageous to nutrient-stressed diatoms by increasing the potential for uptake of scarce nutrients following an intense phytoplankton bloom.

Evolutionary theory predicts that, all else being equal, the genotype producing aggregation behavior will be selected for only if diatoms carrying that genotype remain in

the mixed layer long enough to experience differential growth rates above those of non-aggregating genotypes of the same species. If the magnitude of this differential reproduction is very small, more time will be required to establish the genotype for aggregation within the species. However, as long as the differential reproduction of aggregating diatoms is greater than non-aggregating cells, i.e., the fitness of the aggregation genotype is greater than unity, then aggregation behavior will be selected for and the aggregation genotype will come to dominate the population (see Emlen 1973 for discussion). Phytoplankton cell growth and reproduction is directly related to nutrient uptake. Our finding that aggregated cells may take up nutrients as much as 2.1 times faster than unaggregated cells of the same species suggests that the differential production of aggregated cells has the potential to be quite high. However, even Relative Uptake Factors only slightly greater than unity could result in gradual selection for diatoms which aggregate.

We propose that some aggregating diatoms remain in the mixed layer long enough to experience differential reproduction because of two sequential processes. First, despite their rapid sinking rates, some diatom flocs remain suspended in the mixed layer for many days before they sink out of it. Lande and Wood (1987) calculated that slowly sinking particles may make many vertical excursions within the mixed layer before they are lost via settlement. Empirical evidence indicates that some rapidly settling particles also make such excursions. For example, 5 to 10% of macro-crustacean fecal pellets sinking at rates of 18 to 170 m d^{-1} have been observed suspended in the mixed layer 10 d after production (Alldredge et al. 1987). Moreover, diatom flocs several days old do indeed occur abundantly in surface waters off southern California (Alldredge and Gotschalk in press). Our calculations indicate that cells attached to these flocs, due to increased nutrient uptake, should have a selective advantage over nutrient depleted, unattached cells remaining an equal amount of time in the mixed layer. Second, once replete, at least some cells must fall off flocs before the flocs leave the mixed layer. Diatom flocs are fragile and erode or fragment at shear rates normally encountered in the ocean (McGillivray and Alldredge 1987). Single chains or floc fragments will more easily become suspended than the larger and heavier parent flocs. A cycle of repeated resuspension, coupled with gradual erosion or fragmentation of only a small percentage of diatom flocs formed after a bloom, would be adequate to reseed the mixed layer with unattached vegetative cells replete with nutrients due to their prior association with the flocs. It is these cells which may be capable of sustained survival following a bloom and which eventually re-establish diatom populations in surface waters when more favorable nutrient conditions reoccur.

Acknowledgements. Special thanks to C. C. Gotschalk for field and laboratory assistance in determining the properties of diatom flocs, and G. A. Jackson for reviewing an earlier manuscript. This research was supported by ONR Grant N00014-88-K-0387, and the donors of The Petroleum Research Fund, administered by the ACS, to B. E. Logan, and NSF Grant OCE-85-10826 and ONR Grant N00014-85-K0771 to A. L. Alldredge.

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Date of final manuscript acceptance: February 17, 1989.
Communicated by J. Grassle, Woods Hole

Fractal geometry of marine snow and other biological aggregates

Abstract—Fractal dimensions of aggregates can potentially be used to classify aggregate morphology as well as to identify coagulation mechanisms. Microbial aggregates of *Zoogloea ramigera* have a cluster fractal dimension of 1.8 ± 0.3 (\pm SD), suggesting that these aggregates are formed through cluster-cluster coagulation. An analysis of size-porosity correlations for two types of marine snow aggregates yielded fractal dimensions of 1.39 ± 0.06 and 1.52 ± 0.19 , which were lower than values describing inorganic colloidal aggregation.

Large, amorphous aggregates, known as marine snow, compose the largest component of mass sedimenting through the water column (Fowler and Knauer 1986). These aggregates consist of diatoms, bacteria, fecal

pellets, cast houses of appendicularians, and nearly all other microscopic organic and inorganic matter present in the ocean. The manner in which these larger aggregates are formed is not well understood. The growth of microaggregates (<0.5 mm) and marine snow-sized aggregates (>0.5 mm) can result from Brownian motion, eddy diffusion, shear coagulation, and differential sedimentation, as well as through growth of organisms within the aggregate microhabitat. McCave (1984) has calculated that shear rates in the ocean are too low to account for observed, steady state particle size distributions. Although this conclusion implies that physical mechanisms of aggregate formation are less important than biological mechanisms in marine systems, marine snow aggregates have macroscopic morphologies that are characteristic of inorganic aggregates formed through physical coagulation mechanisms. This similarity, which

Acknowledgments

This work was supported by the Donors of The Petroleum Research Fund (administered by the American Chemical Society) and ONR contract N00014-88-K0387.

can be established with fractal geometry (Mandelbrot 1977; Feder 1988), provides evidence that aggregates can form in the ocean through physical coagulation.

The structures of inorganic aggregates, such as gold particle clusters formed irreversibly in suspension, are scale invariant or fractal (Witten and Cates 1986). The number of particles, N , in a fractal aggregate is

$$N \sim l^{D_n} \quad (1)$$

where D_n is the cluster fractal dimension determined for the object in n dimensions, and l is the characteristic length scale of the aggregate. For a Euclidean object such as a sphere, $D_3 = 3$. Aggregates formed by random processes have fractal dimensions significantly less than the Euclidean 3. Computer simulation of aggregate growth by random processes indicates that the magnitude of the fractal dimension is determined by the mechanism of aggregate growth. Aggregates formed through the addition of particles into the cluster one at a time (particle-cluster) have three-dimensional fractal dimensions in the range of 2.5–3.0 (Schaefer 1989). Aggregates formed through collision of clusters (cluster-cluster) have lower fractal dimensions, typically with $1.6 \leq D_3 \leq 2.2$ (Witten and Cates 1986).

Recent research on inorganic colloidal aggregation has demonstrated a relationship between aggregate structure and cluster-cluster aggregation kinetics (Lin et al. 1989). Computer simulations and experiments with several types of colloids indicate that two different types of aggregates form as a function of different particle stickiness or different collision efficiencies (Table 1). As the probability of attachment on collision approaches unity, particles stick upon contact, forming highly tenuous structures. This pattern results in very rapid coagulation, referred to as diffusion-limited colloidal aggregation (DLCA) because the rate of aggregate growth is limited by particle transport. The highly tenuous structure is a result of very sticky particles moving in a random walk; particles cannot penetrate the aggregate since they attach to particles with which they collide on the aggregate exterior. Aggregates formed through DLCA are char-

Table 1. Fractal dimensions of inorganic colloids formed through either DLCA or RLCA (data from Lin et al. 1989).

Colloid	DLCA	RLCA
Gold	1.86	2.14
Silica	1.85	2.07
Polystyrene	1.82	2.09

acterized by fractal dimensions around 1.8. As the attachment probability approaches zero, particles may collide many times before sticking, allowing them to penetrate the aggregate and increasing the aggregate density. This aggregation is classified as reaction-limited colloidal aggregation (RLCA) and results in aggregates with D_3 values around 2.1.

The occurrence of two distinct regimes of colloidal aggregation suggests that fractal scaling relationships can potentially identify the aggregate formation mechanism as well as the attachment efficiency of particles composing the aggregate. Lin et al. (1989) demonstrated universality of fractal classification for inorganic systems with colloidal gold, silica, and polystyrene. It has not been established, however, that biological aggregates can be similarly classified. Most microorganisms grown in batch culture aggregate during late log growth (Calleja 1984). This aggregation creates a more complex system than previously examined for inorganic coagulation because particles (daughter cells) can be produced during coagulation. Microbial growth occurs during aggregation, so we would expect biological aggregates to have D_3 values that are between values reported for particle-cluster and cluster-cluster models. The size of the microbial aggregates and the range of coagulation mechanisms (e.g. shear, differential sedimentation), however, create conditions far different from systems previously characterized by fractal analysis.

In order to establish that biological aggregates have fractal structures similar to inorganic aggregates, we developed microbial aggregates composed of homogeneously sized particles with pure cultures of microorganisms. We grew aggregates of *Zoogloea ramigera* type I-16-M (ATCC-19623), a rod-shaped, gram-negative bac-

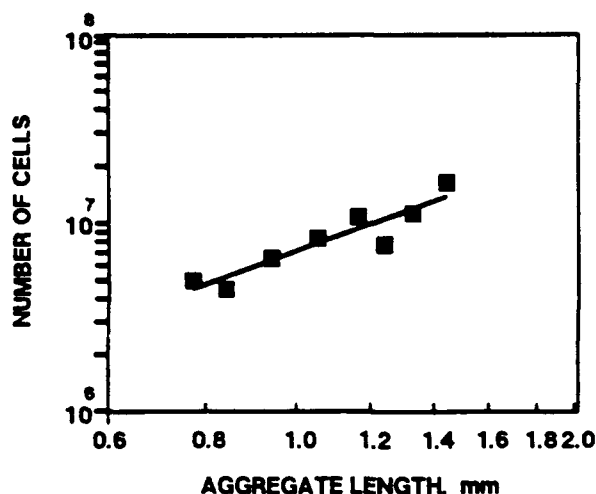


Fig. 1. Cells per floc of *Zoogloea ramigera* (■) with a regression line through the data ($r^2 = 0.87$) indicating $D_3 = 1.8 \pm 0.3$ (± 1 SD). Data points represent averages of 3–6 aggregates.

terium which attaches with cellulosic fibers that resist floc breakage during aggregate-sizing experiments. Suspended cultures (15 ml) were developed at room temperature (23°C) on nutrient broth (Difco) in 36-ml test tubes clamped to a laboratory rotator. After 24 h, cultures were poured into a sterile Petri dish and diluted with sterile water to increase floc separation. Individual aggregates of *Z. ramigera* were captured in ~ 20 μ l of media with a 100- μ l pipette with the plastic tip cut between the 25- and 50- μ l markings and transferred to a 2-ml drop of solution to aid separation of flocs from free-living cells. The pipet tip was then rinsed with sterile water and the aggregate transferred to a well slide. Flocs were viewed with direct light microscopy (10 \times power, Olympus model BH-2) to determine the largest aggregate length and to sketch the aggregate.

The number of cells composing each microscopically sized aggregate was determined by rinsing the slide contents into a sterile, acid-washed vial and adding sterile water for a final volume of 1 ml. The floc was dispersed enzymatically with 10 mg ml⁻¹ of cellulase (Sigma Chemical No. C-7377), vortexing the solution for 1 min, and placing the vials in a rotator. Samples were preserved with 2% formaldehyde and counted with acridine orange epifluorescence (Hobbie et al. 1977). Samples containing incompletely dispersed flocs were discarded.

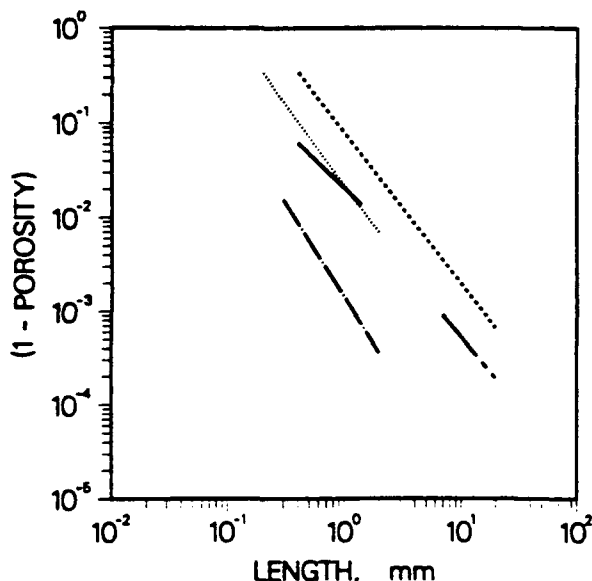


Fig. 2. Occupied volume (1 - porosity) correlations with aggregate size for several different types of biological aggregates from both natural and engineered environments: *Zoogloea ramigera* (—); marine snow (---); diatom flocs (-.-.); normal wastewater bioreactor flocs (.....); filamentous wastewater bioreactor flocs (- - - -).

Transfer of culture material without flocs through the above sequence of steps was used to verify that free-living cells included with the floc transfer were a negligible portion of the total counts.

Fractal aggregates are characterized by large spatial and sample-to-sample fluctuations in properties (Meakin 1988). Therefore, we sized 47 aggregates by longest length and averaged all aggregates within 0.1-mm size classes spanning 0.7–1.5 mm long. From a log-log regression with Eq. 1 and data shown in Fig. 1, we determined a fractal dimension for *Z. ramigera* flocs of 1.8 with a standard error of 0.3 ($r^2 = 0.87$, $n = 8$) based on the longest aggregate length. This value is much smaller than the Euclidean value of $D_3 = 3$ expected for spherical aggregates and is experimental evidence of the three-dimensional fractal structure of these aggregates. The magnitude of this fractal dimension is within the range indicated for aggregates formed through cluster-cluster coagulation, but is not sufficiently precise to identify whether cluster formation is within the range expected for diffusion- or reaction-limited aggregation.

Several investigators have related the po-

Table 2. Fractal dimensions of biological aggregates determined from empirical size-porosity equations.

Biol. aggregate type	Size range (mm)	Fractal dimension	References
Marine snow			
General	0.4–20	$1.39 \pm 0.15^*$	Alldredge and Gotschalk 1988
Diatom	7–20	$1.52 \pm 0.19^*$	Logan and Alldredge 1989
Bioreactor			
Normal	0.2–2	1.3	Tambo and Watanabe 1979
Filamentous	0.3–2	1.0	Tambo and Watanabe 1979
<i>Zoogloea ramigera</i>	0.4–1.4	$1.8 \pm 0.3^*$	This study

* \pm 1SD.

rosity, p , of biological aggregates to aggregate size (Tambo and Watanabe 1979; Alldredge and Gotschalk 1988; Logan and Alldredge 1989) with

$$1 - p = al^b \quad (2)$$

where a and b are empirical constants, and b is negative. Porosity has been determined either directly from gravimetry, assuming an average particle density (Alldredge and Gotschalk 1988; Logan and Alldredge 1989) or indirectly from settling experiments (Tambo and Watanabe 1979). If the number of particles or cells comprising the aggregate is known, the porosity can be directly calculated from

$$1 - p = \frac{NV_c}{V} \quad (3)$$

where V_c is the average volume of cells composing the aggregate and V the volume occupied by an aggregate. Following convention (Witten and Cates 1986; Li and Ganczarczyk 1987; Logan and Alldredge 1989), we defined the volume of *Z. ramigera* aggregates as the volume of a sphere just capable of enclosing the aggregate. Combining Eq. 1 and 3, we obtained the fractal relationship

$$(1 - p) \sim l^{D_3-3}. \quad (4)$$

A comparison of Eq. 2 and 4 shows that $D_3 = 3 + b$. Therefore, the fractal dimension of marine snow, as well as other types of biological aggregates, can be estimated with published size-porosity relationships.

Although marine snow aggregates can be much larger than other types of biological aggregates, they are denser than aggregates of similar size (Fig. 2). The porosities de-

termined for *Z. ramigera* aggregates by our microscopic methods are similar to porosities determined for aggregates normally found in wastewater treatment bioreactors. On occasion, filamentous cells become numerically abundant in these bioreactors. This produces highly filamentous flocs that are morphologically different from normal bioreactor flocs that consist primarily of clumps of spherical or rod-shaped cells. These filamentous flocs are much more porous than normal bioreactor flocs.

The fractal dimensions for both naturally occurring and engineered reactor aggregates are in the range of 1.0–1.5 (Table 2). *Zoogloea ramigera* aggregates grown in the laboratory had the largest fractal dimension of all aggregates examined. This result indicates that aggregates grown in the laboratory are more compact, having higher fractal dimensions than aggregates formed in the ocean.

Fractal dimensions have been calculated with settling data (Li and Ganczarczyk 1989). These fractal dimensions, however, contain a mixture of scaling properties. From a force balance on a settling particle, we have (Bird et al. 1960)

$$V(\rho - \rho_f)g = 0.5A\rho_f C_D U^2 \quad (5)$$

where ρ is the floc density, ρ_f the fluid density, g the gravitational constant, A the projected surface area of the floc perpendicular to direction of settling, C_D a drag coefficient, and U the aggregate settling velocity. For small aggregates, the drag coefficient is proportional to aggregate settling velocity and inversely proportional to aggregate length. For a solid object, such as a sphere with diameter d , $C_D = 24/Re$ for $Re = Ud/\nu \ll$

Table 3. Fractal dimensions of biological aggregates determined from settling velocity data.

Biol. aggregate type	Size range (mm)	Fractal dimension	References
Marine snow (in situ)	7-20	1.26±0.06*	Allredge and Gotschalk 1988
Estuarine	0.02-2	1.78	Gibbs 1985
Lacustrine	0.012-0.04	1.39-1.69	Hawley 1982
Oceanic	~0.1	2.14	
Oceanic	~0.4	1.94	
Recoagulated oceanic sediments	0.1-1	1.57	Kajihara 1971

*±SD.

1 where ν is the fluid kinematic viscosity. Using this drag coefficient in Eq. 5, we obtain

$$U = \frac{V(\rho - \rho_f)d}{12A\rho_f\nu} \quad (6)$$

$$(\rho - \rho_f) = (1 - p)(\rho_c - \rho_f)$$

where ρ_c is the density of the particles comprising the aggregate, so we can define a proportionality between settling velocity and aggregate geometry as

$$U \sim \frac{V(1 - p)d}{A} \quad (7)$$

For a spherical, solid object with $Re \ll 1$, $U \sim d^2$, in agreement with Stokes' law (Bird et al. 1960).

We can define a proportionality for a fractal object by combining Eq. 1, 3, and 7, to obtain

$$U \sim l^{D_3+1-D_2} \quad (8)$$

where we have assumed that $A \sim l^{D_2}$, with D_2 the two-dimensional fractal dimension that relates the projected surface area to the largest aggregate length. Li and Ganczarczyk (1989) have defined the scaling power in Eq. 8 as a single fractal dimension. This scaling power is a function of aggregate geometry in both two and three dimensions and is defined here as a fractal dimension, $D(2)$, without a subscript. The number in parentheses indicates the value of this fractal dimension for a solid object. The fractal dimension D_3 can be estimated with settling velocity data from $D_3 = D(2) - 1 + D_2$, and by assuming that $D_2 \approx 2$.

Values of D_3 obtained from Eq. 8 and settling data span a wide range of 1.26-2.14 (Table 3). Many of these values are below

those expected for cluster-cluster coagulation. Our estimate of D_3 is exact for any self-similar object (Euclidean or fractal) if a geometric length scale is used to define the aggregate area. If longest length is used, then $D_2 \leq 2$, and the calculated value of D_3 is an upper estimate of the true value. Other factors may influence the relationship between D_3 and $D(2)$. For example, the marine snow aggregates examined by Allredge and Gotschalk (1988) extend beyond the creeping flow range and have Reynold's numbers greater than unity. The low value of $D_3 = 1.26$ may result from exceeding the length scales applicable to smaller aggregates. This lower value also may be related to the fact that aggregates examined by Allredge and Gotschalk were examined in situ and therefore were not disrupted by handling. The value of $D_3 = 1.26 \pm 0.06$ (\pm SD) obtained from the settling velocity data of Allredge and Gotschalk is slightly lower than the value of $D_3 = 1.39 \pm 0.15$ (\pm SD) calculated from their gravimetric analysis. This finding suggests that settling velocity correlations may underestimate D_3 values.

Most of the D_3 values calculated for marine snow are smaller than the range of values observed for inorganic aggregates formed through diffusion-limited cluster-cluster coagulation (DLCA). There have been previous reports that biological molecules exhibit different coagulation behavior than inorganic colloids (Feder et al. 1984). At the present time, we have no specific explanation for these results. It is generally accepted that aggregate characteristics are altered by fluid mixing intensity, aggregate age, and other factors not considered in our analysis (Hunt 1986). Computer simulation by Meakin (1988) demonstrated that aggregate

restructuring increases the fractal dimension. In Meakin's study, D_3 increased from 1.89 to 2.13 when long chains of an aggregate contacted and attached to other tenuous structures within the aggregate. Therefore, fluid mixing and turbulence should increase the fractal dimension. Based on our current understanding of the effect of fluid environment and coagulation kinetics, and Meakin's (1988) results, we would expect aggregates in the ocean to have higher fractal dimensions than those measured for cluster-cluster, diffusion-limited coagulation and not the lower values calculated in this study.

We have conducted some preliminary experiments on the effect of fluid environment on the fractal dimension of microbial aggregates. When we cultured *Z. ramigera* in a laboratory reactor (Omni-culture reactor, Virtis Co.) aerated at 1 liter of air min^{-1} and mixed at 160 rpm, the fractal dimension of these aggregates was 3.0 ± 0.4 (\pm SD) (Wilkinson 1989). This fractal dimension is essentially equal to the Euclidean three-dimensional value for a spherical object. This higher fractal dimension may be a result of more aggregate restructuring in a highly mixed vessel than occurs in less turbulent environments, such as the ocean. To date, our experiments have not had well-defined hydrodynamic environments primarily because the environments we are modeling (bioreactors and oceans) are poorly characterized with respect to fluid mixing and turbulence. Our preliminary findings, however, suggest that the fractal dimension of aggregates depends on the fluid mechanical environment.

We conclude that pure and mixed cultures of microorganisms have three-dimensional scaling properties typical of fractal structures. The magnitude of fractal dimensions of marine snow aggregates is lower than the range expected for aggregates formed by Brownian motion of colloidal aggregates and may be related to aggregate restructuring or aggregate formation through other processes, including shear coagulation and differential sedimentation. Although understanding of precisely how these factors influence aggregate characteristics is incomplete, it is hoped that fractal geometry will

provide a basis for studying factors that affect the structure of biological aggregates and aggregation kinetics in natural systems.

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Submitted: 5 July 1989

Accepted: 17 October 1989

Revised: 27 November 1989